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Hamster Female Protein, a Pentameric Oligomer Capable of Reassociation and Hybrid Formation

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ABSTRACT: Syrian hamster female protein (SFP), a serum oligomer composed of five identical subunits, was reassociated in vitro from monomer subunits. The reconstituted pentamer was genuine by morphologic, antigenic, and structural criteria. Another female protein (FP), a homologue from Armenian hamsters (AFP), also reassociated into a pentamer after dissociation with 5 M guanidine hydrochloride. These two FP's hybridized when a mixture of them was dissociated and then reassociated. Differences between the parent FP's were used to show that the recombinant pentamer contained monomer subunits from both SFP and AFP. Reassociation of both FP's was enhanced by increasing FP concentration and also by adding Ca^{2+} during reassembly. The two FP's differed in their reassociation profile in that SFP was especially efficient in reassembly, whereas AFP was more dependent upon Ca^{2+}. Female protein is a homologue of C-reactive protein and amyloid P component, and all of these proteins (pentraxins) share a similar structure. The in vitro dissociation–reassociation of female protein described herein may reflect an in vivo dissociation–reassociation which is functionally important and a common metabolic feature within this family of proteins.

C-Reactive protein (CRP)[1] is an acute-phase protein that was discovered in humans more than 50 years ago (Tillett & Francis, 1930; Abernethy & Avery, 1941). CRP represents a gene that evolved millions of years ago and has been maintained in many species with little change during evolution (Baltz et al., 1982). CRP and a homologous protein, serum amyloid P component (SAP), have been found in most animals and are known to participate in a variety of biological processes [for a review, see Kushner et al. (1982)]. Such conservation of structure and ubiquitous expression suggests that these proteins have an important function; however, a unique role has not been ascribed to them. This family of proteins (pentraxins) shares a common oligomeric structure in which five subunits (≃25 000–30 000 daltons each) are noncovalently assembled into a symmetrical cyclic pentamer (125 000–150 000 daltons) with a characteristic morphology detectable by electron microscopy. Hamster female protein (FP), a recently described member of this family, is a sex-limited homologue that shares many structural and functional features with CRP and SAP (Coe, 1983; Coe & Ross, 1985).

[1] Abbreviations: cpm, counts per minute; CRP, C-reactive protein; FP, female protein; AFP, Armenian FP; SFP, Syrian FP; [A + S]FP, mixture of AFP and SFP homologous pentamers; A*, ^{125}I-AFP; S*, ^{125}I-SFP; (A/S)FP, recombinant heterologous pentamer containing both AFP and SFP monomer subunits; HEA, hen egg albumin; PC, phosphorylcholine; PAGE, polyacrylamide gel electrophoresis; RGG, rabbit γ-globulin; SAP, serum amyloid P component; SDS–PAGE, sodium dodecyl sulfate–PAGE; SDG, sucrose density gradient; Tris-HCl, tris(hydroxymethyl)aminomethane hydrochloride; EDTA, ethylenediaminetetraacetic acid.

Functional studies of pentraxins have naturally focused on the usual pentameric form of the molecule found in serum. Comparatively little is known about the monomer subunit; however, some differences in binding (Gotschlich & Edelman, 1965, 1967) and antigenicity (Potempa et al., 1983) have been reported. Although the relative ease of pentraxin (CRP) dissociation has been known for some time (Gotschlich & Edelman, 1965), this process has not been reversed, as the in vitro reassembly of these pentamers from monomer subunits has not been demonstrated. Many other oligomeric proteins, such as enzymes, however, have been found to readily undergo a reversible dissociation, and this process may be an important mechanism regulating enzyme function (Jaenicke & Rudolph, 1980). If an efficient pentraxin dissociation–reassociation could be documented, it would enhance the notion that such a process also was biologically important for these oligomers. In approaching this problem, we studied the capacity of pentameric hamster female protein (FP) to be reassembled in vitro. The results indicated that a genuine FP pentamer was reassociated from monomer subunits. Furthermore, hybrid pentamers were constructed by the use of two heterologous FP preparations; unique characteristics that distinguished the two parent FP populations were used to clearly document the creation of the hybrid recombinant molecule. This reassociation–hybrid formation was relatively efficient at physiological concentrations of FP and required a preliminary step of salt dissociation.

Materials and Methods

Animals, Antisera, and Purified Proteins. Random-bred Syrian hamsters (*Mesocricetus auratus*), Armenian hamsters (*Cricetulus migratorius*), and New Zealand white rabbits were obtained from the animal facilities of the Rocky Mountain Laboratories. Syrian FP (SFP) was isolated from normal serum, as before, by Ca^{2+}-dependent binding to phosphorylcholine (PC)–Sepharose column and elution with free PC (0.001 M) (Coe et al., 1981). Armenian FP (AFP) was obtained from Armenian hamster serum by similar methods (unpublished). The purity of SFP and AFP was verified by gel diffusion analysis and by polyacrylamide gel electrophoresis (PAGE). Furthermore, when these preparations were used for immunization of rabbits (Coe et al., 1981), the resulting antisera were monospecific. Because of shared antigens between SFP and AFP (unpublished), antisera specific for the homologous FP required absorption by heterologous FP. This was done either by direct absorption (addition, precipitation) or by affinity chromatography with the heterologous FP. Purified FP was labeled with ^{125}I from New England Nuclear (Boston, MA), by using Iodogen (Pierce Chemical Co., Rockford, IL) as previously described (Coe & Ross, 1983).

Analytical Techniques. Agar gel diffusion and PAGE, either with sodium dodecyl sulfate (SDS–PAGE) or without SDS (plain PAGE), were performed as before (Coe et al., 1981). Autoradiography of agar gel diffusions was accomplished by placing the dried slide directly in contact with Kodak Royal Pan Film, whereas fluorography was used with plain PAGE and SDS–PAGE (Bonner & Laskey, 1974). Immune precipitation of ^{125}I-FP was done, as before (Coe et al., 1981), by using a specific rabbit antisera followed by an equivalent amount of guinea pig anti-RGG.

To test the PC binding ability of reassociated (guanidine-treated) SFP, a fraction containing $\simeq$70 000 cpm (0.075 mL) from the 7S region of the sucrose density gradient (SDG) of guanidine-treated ^{125}I-SFP was added to a small (3-mL) column containing 1 mL of PC–Sepharose equilibrated with 0.5 mM $CaCl_2$–0.1 M Tris-HCl. Determinations from pass-through and EDTA (0.1 M) elutable counts per minute (cpm) revealed an 83% recovery of original cpm in the EDTA eluate. Similar results were obtained with free PC (10^{-3} M) elution.

Dissociation–Reassociation Experiments. FP was dissociated with 5 M guanidine hydrochloride because previous data indicated a complete dissociation of CRP (Gotschlich & Edelman, 1965) and FP (Coe et al., 1981) into monomer subunits under these conditions. That is, high-speed sedimentation equilibrium runs of FP in 5 M guanidine hydrochloride–20 mM sodium phosphate revealed a monodisperse homogeneous population of 30 000 molecular weight subunits (Coe et al., 1981). A known amount of ^{125}I-FP ($\simeq 2 \times 10^6$ cpm) was mixed with various concentrations of unlabeled FP (0.02–2.0 mg/mL); this mixture (0.05–0.10 mL) was placed in a small dialysis bag and dialyzed for 18 h at 4 °C against (step 1) 5 M guanidine hydrochloride–20 mM sodium phosphate, pH 7.0, followed by (step 2) a 36-h dialysis against 0.1 M Tris, pH 7.0, with or without $CaCl_2$ (usually 0.001 M). Control samples were dialyzed in a similar fashion except that 0.1 M Tris, pH 7.0, was used instead of guanidine for the first step; therefore, these samples were not dissociated. To determine the molecular form of FP, the total sample after dialysis was carefully overlaid onto a 4-mL 10–37% (w/v) linear SDG and spun at 35 000 rpm for 18 h at 4 °C in a Beckman L2-65B ultracentrifuge equipped with an SW50.1 rotor. Ten microliters of 10 mg/mL solutions of DEAE-purified rabbit γ-globulin [(RGG) Pentrax Biochemicals, Kankakee, IL) and hens' egg albumin [(HEA) ICN, K & K Laboratories, Inc., Plainview, NY) was added to the sample before centrifugation as sedimentation markers; RGG (150 000 daltons) is 7 S, and HEA (43 000 daltons) is 3.5 S. Equal fractions ($\simeq$50 per gradient) were recovered from the bottom of the gradient tube by use of a positive-pressure controlling device. The fractions were assayed for radioactivity in a Beckman γ 4000 automatic γ counter, and the concentration peak of FP, RGG, and HEA in the fractions was determined by ring diffusion analysis using specific antisera. This analytic procedure permitted sufficiently accurate assessment of molecular size from the sedimentation profile so that the difference between pentameric AFP and SFP ($\simeq$10 kilodaltons) could be consistently distinguished (see Figure 4A). The total cpm recovered in the gradient fractions was summated to show recovery efficiency, and the areas corresponding to pentamer ($\simeq$7 S) and monomer ($\simeq$3 S) ^{125}I-FP were expressed as a percent of the original cpm added to the dialysis bag at the beginning of the experiment. The recovery efficiency was rarely greater than 70% and frequently less (especially at low concentrations; see Figure 3) due to denaturation (aggregation etc.) from guanidine dialysis.

The reassembled (post-guanidine) pentameric 7S FP appeared genuine by the following criteria: (1) morphologically; by electron microscopy, the post-guanidine pentamer was identical in appearance with native FP (H. Slayter, personal communication); (2) antigenically; reassembled 7S FP was precipitable by specific antisera in a fashion similar to native FP (Table I); (3) electrophoretically; on plain PAGE and SDS–PAGE, reassembled FP migrated similarly to native FP (Figure 5); and (4) functionally; 7S-reassembled FP was bound (in the presence of Ca^{2+}) and eluted from PC–Sepharose in a manner similar to the parent FP.

The 3S-sedimenting peak of ^{125}I-SFP (found after guanidine treatment) was a monomer (30-kilodalton) subunit, as it was of appropriate sedimentation on SDG and appeared monomeric by autoradiography of plain PAGE (i.e., a fast anodal

smear); on SDS–PAGE fluorograms, the 3S material was typical FP monomer. This 3S ^{125}I-SFP monomer became 7S after another dissociation–reassociation event. Thus, ^{125}I-FP from the 3S area of the gradient was added to FP and again treated with guanidine–Tris or Tris–Tris. Analysis by SDG showed incorporation of ^{125}I-FP into 7S (pentameric) protein, but only after guanidine–Tris treatment. This 7S ^{125}I-FP was completely precipitable with specific anti-FP. On the other hand, only 40–60% of monomeric ^{125}I-FP was precipitable with anti-FP; precipitation assays with monomer were complicated by high background since most monomer cpm adhered nonspecifically to glass surfaces, etc., unless 0.5% NP40 was used.

In the process of doing these dissociation–reassociation experiments, some variability in recovery and in percent reassociation from one experiment to another was apparent; according, in the data presented herein, each figure and graph compares the results from gradients contained within a single experimental group, usually consisting of 12–16 gradients.

In all instances in which hybrids were constructed, the concentration of nonlabeled FP was in great excess (50–200-fold) to the concentration of ^{125}I-FP (FP*). Thus, assuming a random reassociation of the monomer subunits, the composition of any labeled hybrid pentamers would be predominantly (80% or 4:1) that of the nonlabeled FP.

Results

Dissociation and Reassembly of SFP. A constant amount of ^{125}I-SFP was added to a similar volume of purified SFP at three different concentrations (0.04, 0.4, and 4.0 mg/mL). After dialysis against 5 M guanidine and then Tris (or Tris–Tris for controls), the SFP solutions were analyzed by SDG for the size distribution of ^{125}I-SFP. The gradient distribution was 7 S for ^{125}I-SFP (4.0 mg/mL) after control (Tris–Tris) treatment (Figure 1A, top). A similar 7S distribution was found also at the lower control dilutions of SFP (not shown). After treatment of 4.0 mg/mL SFP with guanidine, $\simeq$7S and $\simeq$3S peaks of ^{125}I-SFP were found (Figure 1B). These two peaks represented the reassembled pentamer and monomer of ^{125}I-SFP, respectively. Progressively less pentamer was found as the FP concentration decreased from 4.0 mg/mL (Figure 1B) to 0.4 mg/mL (Figure 1C) to 0.04 mg/mL (Figure 1D). Therefore, the relative proportion of pentamer and monomer was influenced by the concentration of FP during reassociation.

Factors Influencing Recovery and Reassembly. In general, Ca^{2+} is an important ion for CRP, SAP, and FP function (Abernethy & Avery, 1941; Pepys et al., 1977; Coe et al., 1981) and can induce conformational changes in CRP structure (Young & Williams, 1978; Volanakis & Kearney, 1981). Accordingly, the effect of Ca^{2+} on reassociation was tested by adding Ca^{2+} to the Tris dialysis (second step). Duplicate preparations of a low-concentration (0.030 mg/mL) ^{125}I-SFP were dissociated with guanidine and then dialyzed against either Tris-HCl with Ca^{2+} (0.001 M $CaCl_2$) or Tris-HCl without Ca^{2+}. SDG analysis showed the presence of pentameric 7S ^{125}I-SFP with Ca^{2+} dialysis (Figure 2B) and the absence of 7S ^{125}I-SFP without Ca^{2+} dialysis (Figure 2C). Therefore, in this experiment, no pentameric SFP was detected at the low SFP concentration unless Ca^{2+} dialysis was used in the second step. Even more pentamer was reassembled when Ca^{2+} dialysis was combined with $\simeq$10-fold higher FP concentration (0.35 mg) (Figure 2A, top). These results from a single experimental group are depicted in Figure 3; gradients 1–4 show a progressive increase in pentamer re-formation by using higher FP concentration and/or Ca^{2+} concentration. Pentamer yield obtained with 0.001 M Ca^{2+} was better than

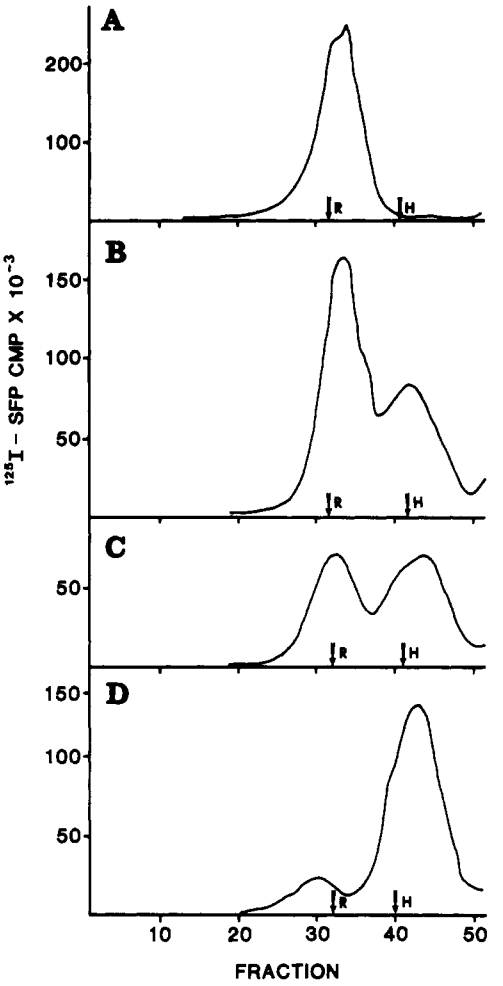

FIGURE 1: Effect of SFP concentration on ^{125}I-SFP reassociation. Distribution of ^{125}I-SFP in 10–37% SDG after prior treatment consisting of Tris dialysis (A) or 5 M guanidine hydrochloride followed by Tris dialysis (B–D) with an SFP concentration of 4.0 mg/mL (B), 0.4 mg/mL (C), or 0.04 mg/mL (D). Bottom of the gradient is on the left; peak concentrations of 7S marker, RGG (R), and 3.5S marker, HEA (H), are noted by arrows. Native pentamer was $\simeq$7 S (A) as was reassociated pentamer (B and C). Monomer SFP was $\simeq$3 S (B–D). Efficiency of pentamer reassociation was directly correlated with SFP concentration.

that with 0.01 M Ca^{2+} (not shown). Note that the total recovery of ^{125}I-SFP cpm (top of bracket, Figure 3, gradients 1–4) also increased as pentamer formation increased. This was a consistent finding and probably due to the relative insolubility of monomer FP and its adhesion to various surfaces. Nevertheless, significant reassembly of pentameric FP was achieved; e.g., in this experiment, 50% of the original ^{125}I-FP (75% of recovered FP) was reassembled as pentamer (gradient 4). In other experiments, up to 72% of the original ^{125}I-FP was reassociated as pentamer (not shown). Actually, this recovery after dissociation was comparable to the recovery of control gradients (Tris–Tris dialysis), which usually yielded only 60–70% of the original FP (not shown). The recovery efficiency of these control (Tris–Tris dialysis) gradients was not affected by the concentration of FP or by the presence of Ca^{2+} in Tris dialysis (not shown).

Hybridization of ^{125}I-SFP with AFP. Armenian hamsters have a serum FP (AFP) that is homologous to SFP (unpublished results). AFP can be distinguished from SFP by differences in antigenicity, electrophoretic mobility, and sedimentation in SDG. Accordingly, if ^{125}I-SFP (S*) were incorporated into a pentamer with characteristics of AFP (A), this hybrid pentameric molecule [(A/S*)FP] would provide

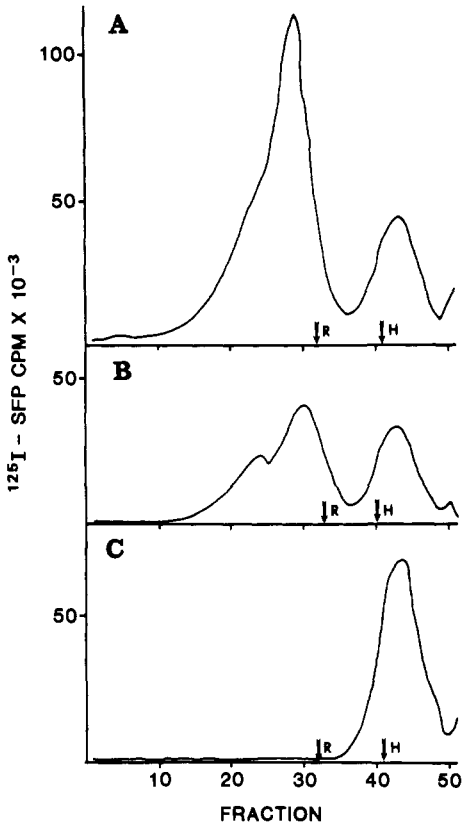

FIGURE 2: Effect of Ca^{2+} dialysis and SFP concentration on ^{125}I-SFP reassociation. Distribution of ^{125}I-SFP in 10–37% SDG after prior guanidine dissociation: (A) 0.35 mg/mL SFP concentration with Ca^{2+} dialysis; (B) 0.03 mg/mL FP with Ca^{2+} dialysis; (C) same concentration as (B) (0.03 mg/mL) but without Ca^{2+} dialysis. In this experiment, no pentamer was reassociated at low concentrations of SFP (C) unless Ca^{2+} was present (B); most reassociation was found using Ca^{2+} with high SFP concentration (A). A population of dimerized pentamers may account for the small peak on the ascending slope of the 7S peak (B).

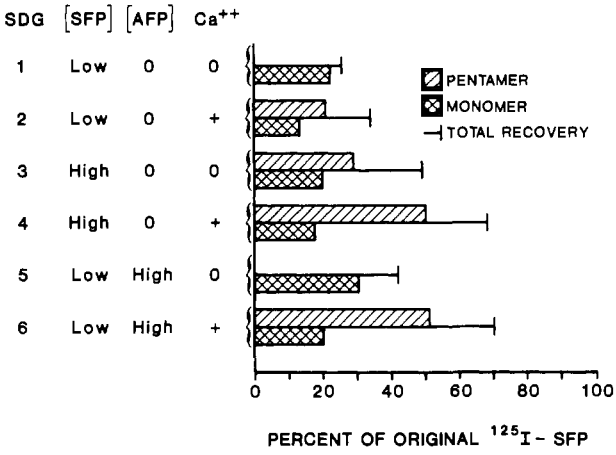

FIGURE 3: Schematic representation of ^{125}I-SFP size distribution (pentamer/monomer) in six SDG's (all one experiment) after guanidine treatment with or without Ca^{2+} dialysis and at various concentrations of SFP (low = 0.03 mg/mL; high = 0.35 mg/mL) or AFP (high = 1 mg/mL). Top of bracket represents total cpm recovered from each gradient as the percent of the original cpm added to that sample at the beginning of experiment.

strong evidence for the compatible reassociation of these molecules. To do this experiment, a low concentration of ^{125}I-SFP (0.03 mg/mL) was added to a high concentration of purified AFP (1 mg/mL) so that the resultant hybrid would be prominently (80%) AFP. After treatment with guanidine and Tris (with or without Ca^{2+}), the samples were examined

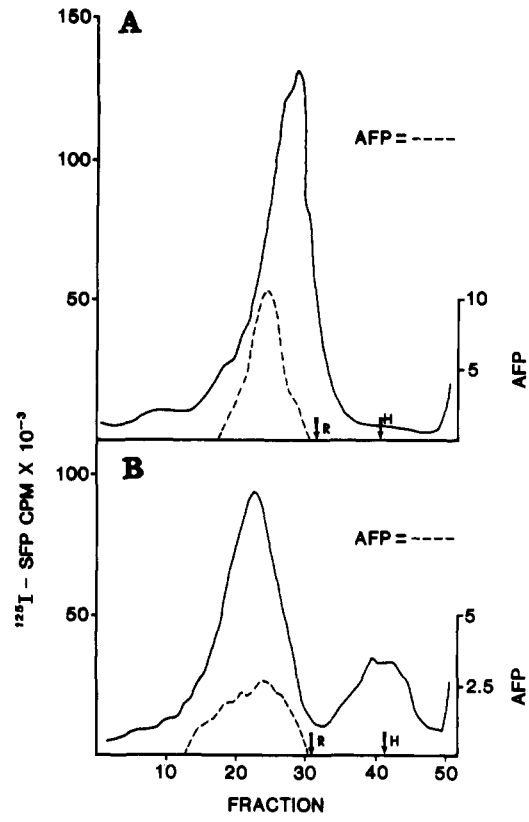

FIGURE 4: Effect of guanidine dissociation on mixtures of AFP and ^{125}I-SFP. Distribution of ^{125}I-SFP in 10–37% SDG when mixtures of low-concentration ^{125}I-SFP (0.030 mg/mL) with high-concentration AFP (1 mg/mL) were either treated by Tris–Ca^{2+} dialysis alone (A, top) or treated by guanidine dissociation followed by dialysis of Tris with Ca^{2+} (B). The protein concentration of AFP in fractions was quantified (---) in (A) and (B). The 7S peak of (B) contains a ^{125}I-SFP–AFP hybrid pentamer primarily composed of AFP subunits.

by SDG in the usual way. Figure 4 (A, top) shows the gradient analysis of the control FP (Tris–Tris) dialysis compared to (B) guanidine-treated FP with subsequent Ca^{2+}–Tris dialysis. From Figure 4B, it is apparent that when a high concentration of AFP was present with a low concentration of ^{125}I-SFP during reassociation, a significant amount of 7S ^{125}I-labeled pentamer was reassociated. To show that this labeled 7S pentamer (Figure 4B, fraction 23) was a hybrid (A/S*)FP, vs. a mixture of (A + S*)FP as in Figure 4A (fraction 28), we looked for the presence of labeled S* with AFP characteristics. For example, on a plain PAGE fluorogram (Figure 5A), native AFP migrated distinctly slower than SFP. Note that the hybrid 7S pentamer (A/S*)FP of Figure 4B gradient also migrated slower than ^{125}I-SFP and similar to ^{125}I-AFP. On SDS–PAGE (Figure 5B), however, this same hybrid was dissociated so that the monomer S* component migrated faster, like a typical independent SFP monomer; SFP monomer is $\simeq$2 kilodaltons smaller than AFP monomer (unpublished results). In contrast, examination of the 7S ^{125}I-pentamer in Tris control (Figure 4A) revealed a normal SFP mobility of the radiolabeled SFP (not shown).

Another method used to show recombinant pentamer was by immunoprecipitation. Antisera specific for unique antigenic determinants of AFP were tested for their capacity to precipitate the ^{125}I-SFP in the 7S gradient peak of the hybrid (Figure 4B) vs. the mixture (Figure 4A). As seen in Table I, rabbit antisera that were specific for AFP after absorption with SFP could not precipitate native ^{125}I-SFP when alone or when mixed with AFP, as in the Tris control mixtures [(S* + A)FP]. When S* was recombined with A as a hybrid

Table I: Immune Precipitation of ^{125}I-Labeled Pentamer FP after Guanidine or Tris Treatment of ^{125}I-SFP in the Presence of Excess SFP or AFP[a]

rabbit sera	^{125}I-SFP + SFP + guanidine → [S*/S]FP	^{125}I-SFP + AFP + Tris → [S* + A]FP	^{125}I-SFP + AFP + guanidine → (S*/A)FP
anti-SFP	97, 98[b]	99, 100	87, 97, 99, 100
anti-SFP-specific (absorbed with AFP)	95, 99	93, 95	89, 92
anti-AFP-specific (absorbed with SFP)	2, 5, 17	3, 5, 12, 14	90, 92, 94, 96, 96
NRS[c]	3	1	1

[a] ^{125}I-SFP in the presence of a 50–150-fold excess of AFP or SFP was treated with Tris or guanidine and then fractionated on SDG. The pentameric FP (7S region of gradient) was tested for immune precipitation of ^{125}I-SFP by various antisera. Aggregate results from two separate experiments each with appropriate controls are presented. [b] Percent ^{125}I-FP pentamer immune precipitated by given antisera. [c] NRS = normal rabbit serum.

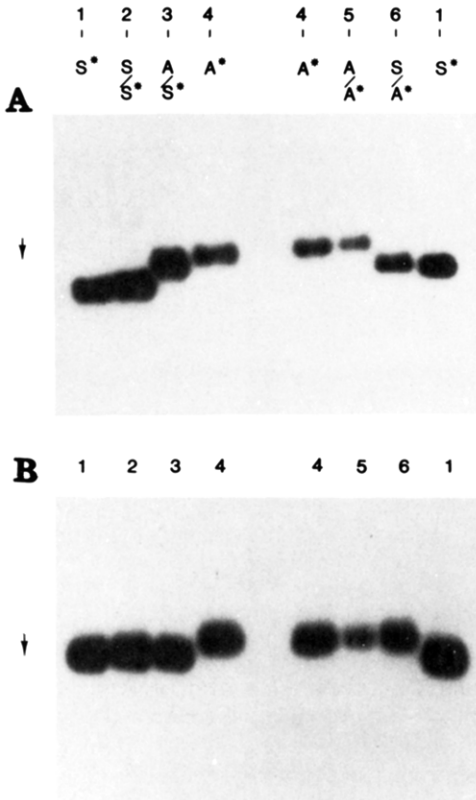

FIGURE 5: Plain PAGE (A) and SDS–PAGE (B) fluorograms of 7S ^{125}I-FP pentamers obtained from the 7S peak of SDG. The pentamers represented are ^{125}I-SFP and ^{125}I-AFP and hybrids made with unlabeled heterologous FP. Lanes 1 and 4 represent native ^{125}I-SFP (S*) or ^{125}I-AFP (A*), respectively, without dissociation (Tris dialysis). Lanes 2 and 5 represent reassociated homologous ^{125}I-SFP (S/S*) or ^{125}I-AFP (A/A*) pentamer formed after guanidine treatment, which has an electrophoretic mobility similar to native preparations. Lane 3 represents hybrid pentamers of S* made with excess AFP (A/S*) so that S* migrates on plain PAGE as AFP. Lane 6 represents a hybrid made of A* with excess SFP (S/A*) so that A* migrates on plain PAGE as SFP. When these same preparations were analyzed on SDS–PAGE (B), the ^{125}I component of the hybrid (lanes 3 and 6) was monomeric and had a mobility similar to the parent monomer. AFP monomer was ≃2 kilodaltons larger than SFP. Direction of migration = top to bottom.

molecule (S*/A)FP, however, ^{125}I-SFP was precipitated by specific anti-AFP.

The reaction of specific anti-AFP with ^{125}I-SFP hybrid is shown more graphically in the autoradiograph of the gel diffusion (Figure 6A). Specific anti-AFP did not precipitate SFP in whole Syrian hamster serum (WS) and also did not precipitate ^{125}I-SFP in mixtures with AFP [(A + S*)FP]. However, when ^{125}I-SFP was hybridized to AFP [(A/S*)FP], the ^{125}I-SFP was coprecipitated. Therefore, mixtures of the

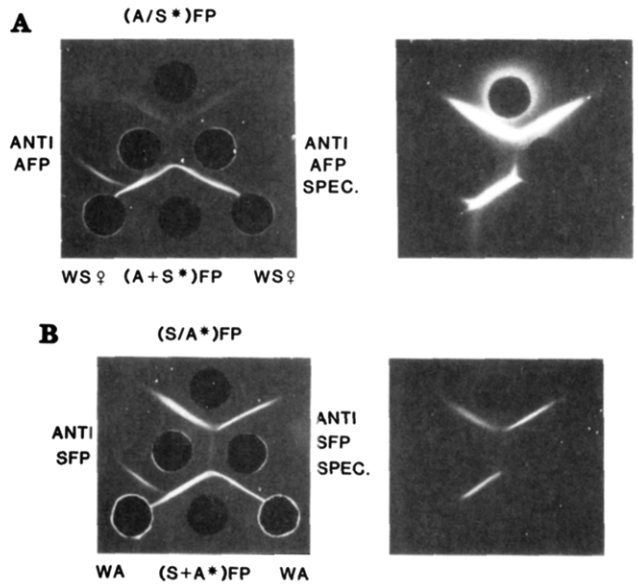

FIGURE 6: (A) Presence of ^{125}I-SFP and AFP in the same pentamer after guanidine dissociation–reassociation. Gel diffusion analysis of 7S (pentamer) peak from SDG separation of ^{125}I-SFP and AFP mixtures after guanidine treatment, (A/S*)FP, or Tris dialysis only, (A + S*)FP. Before absorption with SFP, anti-AFP unabsorbed (left) precipitates SFP in whole female Syrian serum (WS) but not after absorption (anti-AFP spec) (right). The autoradiograph of this gel on the right shows that specific anti-AFP does not precipitate S* in mixtures with AFP [(A + S*)FP] (bottom) but does precipitate S* incorporated in hybrid molecules, (A/S*)FP. (B) Gel diffusion analysis of the 7S (pentamer) peak from SDG separation of ^{125}I-AFP and SFP mixtures after guanidine treatment [(S/A*)FP] or after Tris dialysis alone [(S + A*)FP]. Anti-SFP precipitates AFP in whole Armenian (WA) sera before (left) but not after absorption with AFP (right, anti-SFP spec). The autoradiograph of the gel shows specific anti-SFP only precipitates A* when hybridized (S/A*) with SFP after reassociation.

AFP and SFP did not hybridize unless preceded by guanidine dissociation.

Another indication of hybridization could be seen on SDG analysis, because native AFP sedimented slightly lower in gradient than SFP, consistent with the larger monomer size of AFP seen on SDG–PAGE. This difference in sedimentation of the two FP's was apparent by examination of AFP + ^{125}I-SFP mixtures after simple dialysis (Figure 4A). After hybridization (Figure 4B), the distribution of ^{125}I-SFP was lower in gradient and symmetrical with the AFP peak. The presence of an AFP 7S peak in Figure 4B also indicated that AFP could reassociate after guanidine treatment. The results of Figure 4B are graphically presented in Figure 3 (gradient 6) and show that 51% of the original ^{125}I-SFP was recovered as hybrid pentamer. This reconstitution was similar to homologous SFP recovery (50%) at high (0.35 mg/mL) con-

centration (Figure 3, gradient 4). Both of these reassociations were optimized with Ca^{2+} dialysis; if Ca^{2+} was not present, homologous reassembly (Figure 3, gradient 3) was decreased to 29%, and heterologous reassembly was absent (Figure 3, gradient 5). Thus, AFP reassociation was more Ca^{2+} dependent than SFP reassociation.

Studies on Dissociation–Reassociation of ^{125}I-AFP. The capacity of AFP to reassociate after guanidine was indicated in the previous results when ^{125}I-SFP was used. Thus, AFP in high concentrations was able to "rescue" low concentrations of ^{125}I-SFP during guanidine dissociation, and the resultant 7S ^{125}I-labeled pentamer had antigenic and physical characteristics of AFP. Furthermore, a 7S peak of AFP was detected by a ring diffusion assay after guanidine treatment of AFP. Reassociation of AFP appeared to be more Ca^{2+} dependent than that of SFP. To confirm and expand these findings, ^{125}I-AFP was used directly to observe AFP reassociation and determine ^{125}I-AFP capacity to hybridize when reassociated in the presence of an excess of nonlabeled SFP.

In general, recovery of control (Tris–Tris dialysis) ^{125}I-AFP and reassociation of ^{125}I-AFP were less efficient than SFP; at low concentration (0.02 mg/mL), ^{125}I-AFP did not re-form pentamer, even in the presence of Ca^{2+}. At higher concentration (1.0 mg/mL), a 20% pentamer recovery after dissociation was realized, and no reassembly was detected without Ca^{2+} dialysis (not shown).

However, ^{125}I-AFP could be reassembled without Ca^{2+} dialysis if ^{125}I-AFP was reassociated in the presence of excess SFP. A more efficient reassembly of ^{125}I-AFP with SFP was found when Ca^{2+} was used for dialysis. Under these conditions, recovery of ^{125}I-labeled pentamer was more efficient when heterologous SFP was used (48% yield) than when homologous AFP was used (20% yield), again demonstrating the especially effective reassociation of SFP.

Similar to previous results, the presence of ^{125}I-AFP/SFP hybrid [(A*/S)FP] was seen only after dissociation and detected by: (1) a change in ^{125}I-AFP SDG sedimentation (to SFP position); (2) a faster electrophoretic mobility (to SFP position) of the 7S ^{125}I-AFP hybrid on plain PAGE (Figure 5A) and reversion to normal ^{125}I-AFP monomer mobility on SDS–PAGE (Figure 5B); and (3) immune precipitation (95%) of the 7S ^{125}I-AFP hybrid by specific anti-SFP (absorbed to excess with AFP). The specificity of this latter reaction is shown in Figure 6B.

Discussion

The results of the present study indicate that pentameric hamster female protein can be reassembled in vitro from monomers and that these constructs are indistinguishable from native pentamer. This reassociation was a consistent phenomenon, and although the efficiency would vary from experiment to experiment, most of protein could be harvested as pentamer when SFP was at high concentration, i.e., comparable to that found in the serum of normal female Syrian hamsters (1–3 mg/mL) (Coe, 1977). When Ca^{2+} was present during reassociation, the capacity for pentamer formation was enhanced, and effective reassociation was found also at low concentrations of FP, similar to levels found in the serum of normal male Syrian hamsters (0.015 mg/mL) (Coe & Ross, 1983).

The effect of Ca^{2+} was not unexpected, considering the importance of this ion for CRP, SAP, and FP ligand binding functions (Abernethy & Avery, 1941; Pepys et al., 1977; Coe et al., 1981). CRP subunits have Ca^{2+} binding sites (Gotschlich & Edelman, 1967), and addition of Ca^{2+} induces structural changes in CRP (Young & Williams, 1978; Volanakis & Kearney, 1981). The enhancing effect of Ca^{2+} on FP monomer reassociation may be the result of a corresponding change in FP structure. Possibly Ca^{2+} stabilizes FP pentamer, as suggested by studies on human CRP (Potempa et al., 1983). At present, the specificity of Ca^{2+} on FP reassociation is unknown. Other ions may be equally or more effective. However, the use of physiological levels of Ca^{2+} was advantageous in documenting that SFP would reassociate at low protein concentration. Furthermore, Ca^{2+} appeared to be an obligate requirement for AFP reassembly, so that these two similar FP's were easily distinguished by their Ca^{2+} requirements for reassociation.

Monomeric FP was relatively insoluble in Tris buffer solution and adhered nonspecifically to various surfaces; the overall recovery of original cpm in the SDG after guanidine treatment was low (10–20%), unless pentamer reassociation had taken place. It is possible that this insolubility (hydrophobicity?) of monomeric FP promoted the reassociation after guanidine dissociation. Pentamer reassociation was favored by higher FP concentration, with the result that trace amounts of ^{125}I-FP could be "rescued" by addition of nonlabeled FP. This "rescue" was not enhanced (or hindered) when other proteins (HEA, RGG, or bovine serum albumin) were present during guanidine dialysis (not shown). Another heterologous FP, however, could contribute its subunits to promote this reassociation so that hybrid pentamers were assembled with an efficiency similar to the homologous construct. Indeed, more reassembled pentamer was found with ^{125}I-AFP by hybridization with excess SFP than by combining ^{125}I-AFP with excess homologous AFP; in the absence of Ca^{2+}, hybridization with SFP was the only way to reassociate ^{125}I-AFP. This result indicates that the characteristics of reassembly and recombinant molecules were dictated by the most prevalent FP in the mixture, which in this study was the unlabeled FP.

The ease of SFP and AFP hybridization indicates a certain conservation of structural features required for recombination. The primary sequence of SFP and AFP is probably quite similar; even human SAP, a taxonomically distant homologue, shares 69% of identical sequence with SFP (Dowton et al., 1985). Because pentraxins in general have a relatively similar structure–primary sequence, one might expect that such dissociation–reassociation will be found within other members of this family, even though differences in stringency and ionic requirements for reassociation undoubtedly exist, as seen here with AFP and SFP. Also, conditions for dissociation will need to be tailored to the individual protein; for instance, some pentraxins have been noted to undergo irreversible denaturation by 5 M guanidine treatment (unpublished observations). Recombinant studies using a cooperative pentamer like SFP for the framework of the hybrid may be useful to probe for similar reassociation features expressed on more distantly related pentraxins.

In this study, FP was dissociated with guanidine, because this treatment was known to completely monomerize FP (Coe et al., 1981). Although successful reassembly of monomer was found after this rigorous salt treatment, the calculated percent recovery of the original ^{125}I-FP indicated significant losses (30–75%, depending on FP concentration; see Figure 3) due to aggregation, insolubility, etc. An efficient reversible dissociation (and hybridization) of FP should be feasible with more physiological dissociation systems especially those involving hydrophobic conditions. Hybridization was never observed with simple mixtures of heterologous FP's when left together at 4 °C in Tris buffer for a prolonged interval (data not shown). Therefore, under these conditions, either disso-

ciation was not reversible or the rate of dissociation was too slow for detection. We favor the latter explanation because purified FP was not lost from solution during extended 4 °C storage and FP monomer produced by various techniques has been consistently insoluble. The apparent stability of purified FP in physiological solution appears different from many oligomeric proteins, which are in a constant state of dissociation and reassociation and, in some cases, hybridization when allelic forms are expressed (Schulz & Schirmer, 1979; Nichol, 1981; Banaszak et al., 1981). Many of these oligomers are intracellular enzymes and are usually aggregates of dimers or trimers with very efficient reassociation characteristics (Jaenicke & Rudolph, 1980; Banaszak et al., 1981; Lauffer, 1978). FP and pentraxins in general may represent oligomers with unusual stability in the aqueous environment; this condition would be advantageous for an extracellular serum protein made up of subunits which are subject to kidney excretion as they are smaller than renal threshold (50 000 daltons) (Putnam, 1985).

Little information is available about monomer function in pentraxins, as tests for monomer function require an appropriate solute for solubility, nondenaturing conditions for dissociation, etc. The reassembly in vitro of a pentraxin as demonstrated herein indicates the potential for a reversible dissociation of these oligomers in an appropriate environment in vivo. Perhaps the monomer has a significant functional role, for example, within lipid environments not accessible to the pentamer. This hamster FP hybrid model may provide an opportunity to assess the extent and dynamics of pentraxin reassociation in vivo.

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