MODULATION OF FIBRIN CLOT FORMATION BY HUMAN SERUM AMYLOID P COMPONENT (SAP) AND HEPARIN*

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Serum amyloid P component (SAP)[1] is a normal plasma glycoprotein in man with a circulating concentration of ~40 μg/ml (1), and is probably identical to the amyloid P component associated with most forms of amyloid deposits (2). Ligands identified thus far for SAP include agarose (3–5), heparin (6, 7), heparin sulfate bound to Sepharose 4B (8), isolated amyloid fibrils (9), zymosan (9, 10), iC3b and C4 binding protein (11–13), and fibronectin (14). Recently, it was shown that an apparent tissue counterpart of SAP is an integral constituent of normal vascular basement membranes (15), and is distributed throughout the peripheral microfibrillar mantle of elastic fibers in normal skin and blood vessels (16). Moreover, supraphysiological concentrations of SAP (150–300 μg/ml) have been reported to affect blood coagulation (17).

SAP-like proteins have been isolated from the sera of elasmobranchs, teleost fish, amphibia, and mammals (18). These proteins resemble human SAP in partial amino acid sequence (reviewed in reference 19) and calcium-dependent ligand binding specificity. The stable conservation in SAP during much of vertebrate evolution implies that SAP may have an important function(s). Indeed, SAP is a member of a super family of structurally related proteins termed pentraxins (20) and shares a number of physiochemical properties with the acute-phase reactant, C-reactive protein (CRP), although unlike CRP, SAP is not an acute phase reactant in man.

The present study systematically investigates the effect(s) of SAP on blood coagulation, and provides evidence for an interaction between SAP and certain major components of the coagulation system. A potential function for SAP in host defense is suggested.

Materials and Methods

Preparation of Normal Plasmas. Blood obtained from healthy volunteers was anticoagulated with acid-citrate dextrose (22°C), platelet-rich plasma (PRP) obtained, and the

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[1] *Abbreviations used in this paper*: APTT, activated partial thrombopolastin time; AT III, antithrombin III; CRP, C-reactive protein; FPA, fibrinopeptide A; PAGE, polyacrylamide gel electrophoresis; SAP, serum amyloid P component; SDS, sodium dodecyl sulfate; RIA, radioimmunoassay; RID, radialimmunodiffusion; RVV, Russell's Viper Venom; TRIS-Ca^{++}, Tris-saline buffer containing 2 mM $CaCl_2$, pH 7.4.

final platelet-free plasma pools prepared from PRP by centrifugation at 2,000 *g* for 20 min (11, 21). The pools were stored at −20°C before use.

Isolation of Serum Amyloid P Component. SAP was isolated (4, 21) as previously described with final preparations dialyzed against 1,000 volumes of buffered saline, pH 7.2; concentration was determined by radialimmunodiffusion (RID), absorbance at 280 nm using an average $E^{1\%}_{1cm}$ = 18.2 (22) and Lowry analyses (23). Purity was assessed by polyacrylamide gel electrophoresis (PAGE) in the presence of sodium dodecyl sulfate (SDS) and urea (20, 21, 24). Application of 100 μg SAP to SDS-PAGE resulted in a single detectable protein species; (sensitivity: ≪2 μg protein; purity: ≥98%). To further assure purity, we labeled SAP with ^{125}I, as described (21), and re-analyzed the preparations with SDS-PAGE: >99% of the radiolabel was found associated with an electrophoretic species corresponding to the subunit molecular weight of SAP in SDS-PAGE. C-reactive protein (CRP), an acute phase homologue of SAP in man (20), was not detected in these preparations of SAP as adjudged by RID; SAP was applied at concentrations of 600–1,200 μg/ml, resulting in a nominal sensitivity of <1 μg CRP antigen per 200 μg SAP. The purified SAP was filter sterilized (Millipore Corp., Boston, MA) and stored at 4°C. The average yield was 44%.

Activated Thrombofax Clot Time (Activated Partial Thromboplastin Time; APTT). Plasma (0.2 ml) was pipetted into a glass tube at 37°C. Thrombofax Reagent (0.2 ml; Ortho Diagnostics, Raritan, NJ) and 0.14 ml of standard buffer (0.05 M Tris-HCl, 0.15 M NaCl, pH 7.4) were added and incubated for 5 min, the fibrin clot induced by recalcification, and the clot time determined (25, 26). In experiments evaluating the effect of SAP and/or heparin on Activated Thrombofax clotting time, SAP was premixed with buffer; heparin (beef lung, obtained from the Upjohn Pharmaceutical Co., Kalamazoo, MI, as drug #U-1394, Lot #730-EH) was added 1 min before the addition of $CaCl_2$.

Tissue Thromboplastin Clot Time (Prothrombin Time). To plasma (0.2 ml) prewarmed to 37°C in a plastic tube was added 45 μl of standard buffer or SAP, and 40 μl of heparin or saline, and the mixture was incubated (1 min; 37°C); next, 90 μl each of thromboplastin reagent and $CaCl_2$ (at 20 mM) were added and clot time was recorded.

Russell's Viper Venom (RVV) Clot Time (27). Standard buffer (0.3 ml), or SAP, was mixed with 200 μl prewarmed plasma and incubated (1 min; 37°C). Heparin (25 μl) and additional standard buffer (35 μl) were then added, and after 1 min, 105 μl each of RVV (prewarmed at 37°C) and 20 mM $CaCl_2$ were introduced into the tube.

Activated Factor X Clot Time (from Mann; 28). Before the addition of 50 μl Factor Xa to a plastic tube, 0.1 ml of plasma and 0.35 ml standard buffer were incubated at 37°C for 1 min (in test samples, SAP and/or heparin were also included). Exactly 90 s following Factor Xa addition, 0.1 ml was transferred into a second tube at 37°C; 20 s later, 0.1 ml of 20 mM $CaCl_2$ was added to the second tube and clot formation initiated by addition of 0.2 ml plasma-cephalin.

Thrombin Clot Time (29). 200 μl plasma (37°C) was mixed for 1 min with 0.15 ml standard buffer containing various amounts of SAP/heparin and 0.1 ml saline. Next, 50 μl thrombin was added and the clot time measured.

Fibrinogen / Thrombin Clotting Assay. Various concentrations of thrombin were added to human fibrinogen (2 mg/ml) in the presence of different amounts of SAP/heparin. Total volume was 0.5 ml in 10 mM Tris-saline buffer containing 2 mM $CaCl_2$, pH 7.4 (Tris-Ca^{++}).

Radioimmunoassay of Fibrinopeptide A (30). A radioimmunoassay (RIA) for measuring fibrinopeptide A (FPA) was purchased from Mallinckrodt (St. Louis, MO). Fibrinopeptide A was generated as follows: 0.1 ml of a plasma pool was pipetted into a plastic microfuge tube and diluted to 0.45 ml with Tris-Ca^{++} buffer. FPA release from fibrinogen was initiated by addition of 50 μl thrombin (0.1–0.2 U/ml); 1 min later, the tubes were centrifuged (1 min; 4°C), and the supernatant aspirated and mixed with 1 ml of Bentonite slurry. The supernatant containing FPA was re-collected and tested. To determine the effects of SAP and heparin on the production of fibrinopeptide A, SAP and heparin were preincubated with the diluted plasma sample before the thrombin addition.

Fibrin Polymerization. Fibrin polymerization was performed according to the method of Hall and Slayter (31).

Results

Effect of SAP on Extrinsic, Intrinsic, Common, and Terminal Phases of the Coagulation Cascade. SAP (5–125 μg/ml) had no effect on the clot times generated by activating the intrinsic pathway with Activated Thrombofax reagent in the absence of heparin (Fig. 1). However, in the presence of heparin, which by itself had only a minor anticoagulant effect, SAP greatly prolonged the onset of clot formation. No clot formation was observed when the SAP concentration used was increased beyond 100 μg/ml.

In the absence of heparin, SAP (10–60 μg/ml) did not affect the fibrin clot time obtained by initiating the intrinsic coagulation pathway with tissue thromboplastin reagent, as depicted in Fig. 2. However, in the presence of heparin, SAP again greatly prolonged onset of clot formation; no clot formation was observed when the SAP concentration used exceeded 60 μg/ml.

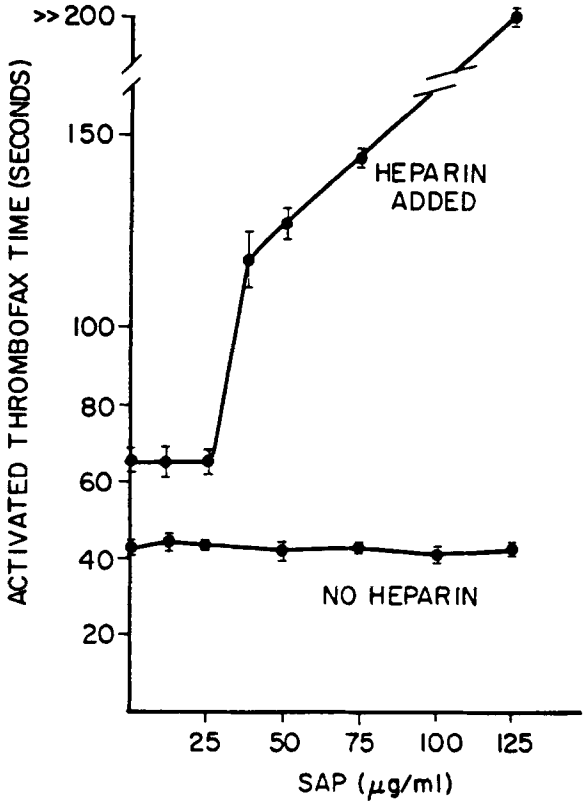


FIGURE 1. The comparative effect of SAP on clot times (± SD) generated by Activated Thrombofax reagent in normal plasma in both the presence or absence of heparin. In Figs. 1–4, the concentration of heparin used was determined by pretitration of each plasma pool during the respective experiment. The concentration of heparin was that amount which increased clotting times by 20–25 s above baseline levels in the absence of SAP. Final concentrations ranged between 0.4 and 1.0 NIH U/ml heparin.

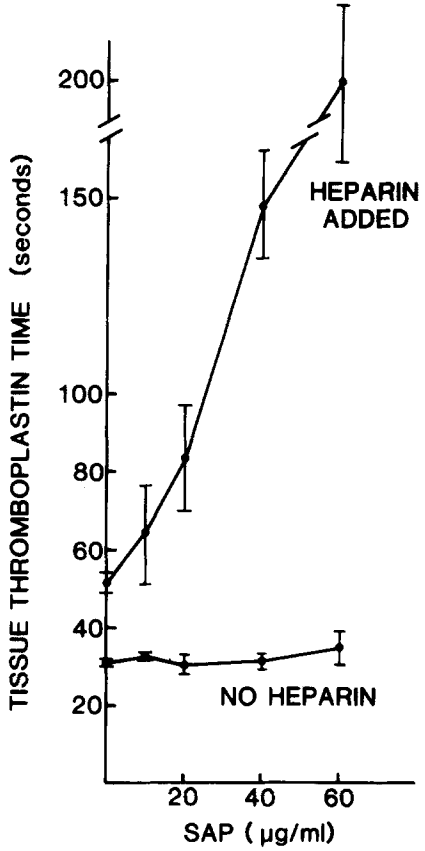


FIGURE 2. Effect of SAP on clot times (± SD) generated by Tissue Thromboplastin in normal plasma both in the presence or absence of heparin (see Fig. 1).

The potent anticoagulant activity of various concentrations of SAP in the RVV assay in the presence of heparin is shown in Fig. 3. As RVV initiates the common coagulation pathway at the level of Factor X, this result, together with results from Figs. 1 and 2, show that in the presence of heparin each the intrinsic and extrinsic pathways were affected by small amounts of SAP (<40 μg/ml). Similar studies revealed that SAP alone (10–45 μg/ml) did not affect clot times generated by the direct addition of activated Factor Xa and cephalin. However, in the presence of heparin, SAP again exhibited an anticoagulant activity.

To test the effect of SAP and heparin on the terminal stage of the coagulation system, a thrombin time assay was performed. Fig. 4 demonstrates that in the absence of heparin, SAP (5–35 μg/ml) failed to prolong the clot time. However, SAP prolonged clot times in presence of heparin. The threshold concentration of SAP required in this system was found to be 20–25 μg/ml, and resulted in the absence of clot formation.

These results demonstrated that in the presence of minimal amounts of heparin, fibrin clot times generated by activation of both the intrinsic and extrinsic pathways were prolonged by SAP, with major sensitivity to the presence

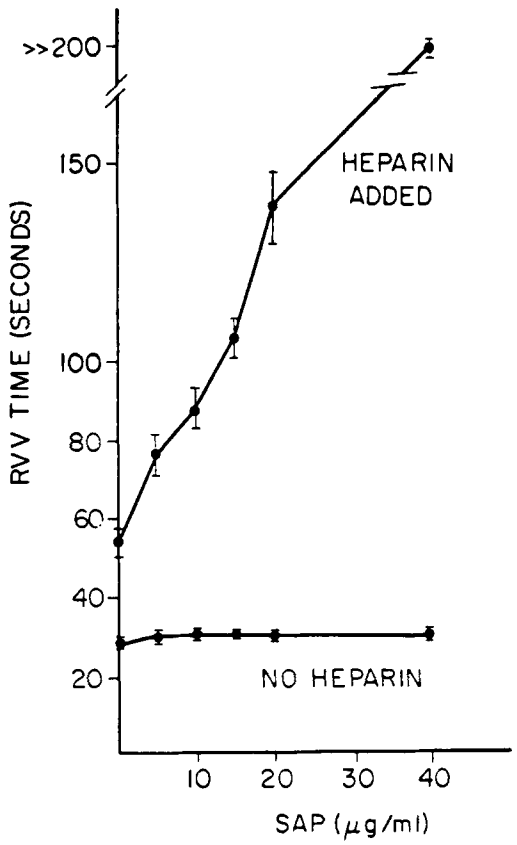


FIGURE 3. Effect of SAP on clot times (± SD) generated by Russell's Viper Venom-cephalin in the presence or absence of heparin (see Fig. 1).

of SAP observed at the distal level of the coagulation system. Additional studies ruled out that the anticoagulant synergism between SAP and heparin was the result of potentiation of anti-thrombin III (AT III) activity as compared to the effect of heparin alone; this inhibition of clot formation also occurred in plasma genetically deficient in coagulation Factor XIII, negating an affect upon Factor XIIIa-driven trans-amidation reactions as the mechanism of action. SAP and heparin also failed to alter the euglobulin clot lysis time (32) or the kinetics of ^{125}I-fibrin(-ogen) release from fibrin clots digested with plasmin (Fig. 5; reference 32). Thus, the prolongation of fibrin clot times by SAP and heparin was not a reflection of an enhanced rate of fibrinolysis.

Evaluation of the Role of SAP in the Conversion Process of Fibrinogen to Fibrin. The terminal phase of coagulation, i.e. the conversion of fibrinogen to fibrin (34), is classically described as composed of several reactions (35). First, thrombin cleaves two pairs of peptides from fibrinogen, termed fibrinopeptide A and B (released from the N-terminal of the α and β chains, respectively; reference 36); second, upon release of these peptides, polymerization of the fibrin monomer units occurs spontaneously. Table I illustrates the effects of SAP and heparin on thrombin clot times and the release of fibrinopeptide A (FPA) as a marker of

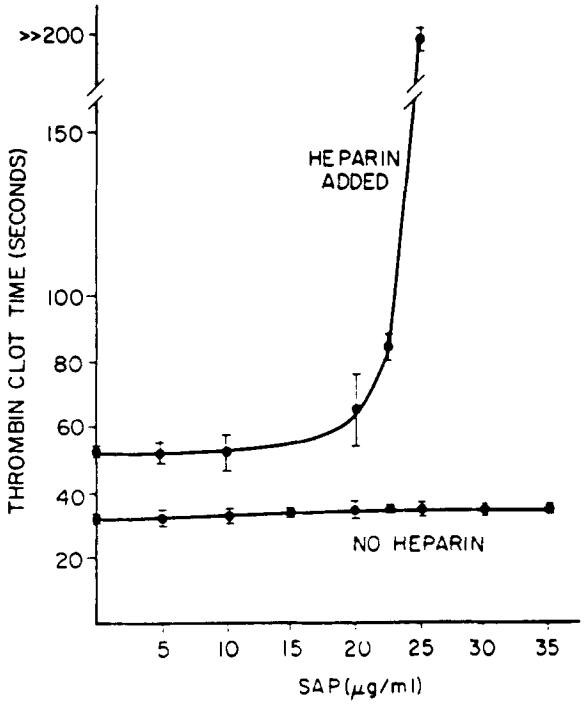


FIGURE 4. The comparative effect of SAP on clot times (± SD) generated by addition of thrombin to normal plasma in the presence or absence of heparin (see Fig. 1).

fibrinogen cleavage in plasma, and the proteolytic activity of thrombin.

SAP (50 μg/ml) did not affect the proteolytic action of thrombin measured either as release of FPA or liberation of p-nitroaniline from the chromogenic substrate; when compared with controls, 50 μg/ml SAP also failed to demonstrate any anticoagulant activity. However, SAP at 200 μg/ml reduced the production of FPA to 75% without any direct inhibition of thrombin activity, and this was associated with a prolonged clot time. Heparin delayed the thrombin clot time from 23.9 to 48.2 seconds, which correlated with the reduction of FPA production in plasma to 60%. In combination, SAP (50 μg/ml) and heparin synergistically delayed fibrin clot formation, and further decreased FPA production. These results suggest one possible mechanism by which SAP may express its anticoagulant capacity.

Similar experiments were carried out in a purified system composed of human fibrinogen, thrombin, SAP, and heparin; results are shown in Table II. SAP alone (80–150 μg/ml) failed to prolong the thrombin clot time or interfere with the production of FPA. Heparin alone slightly delayed development of the fibrin clot but did not alter FPA release. In combination, SAP and heparin synergistically (but minimally) prolonged the onset of fibrin formation, but did not interfere with the release of FPA. These data are in stark contrast to the results observed in plasma (Figs. 1–4, Table I).

The substantial differences observed between plasma and the purified component system suggested that additional plasma constituent(s), perhaps a natural

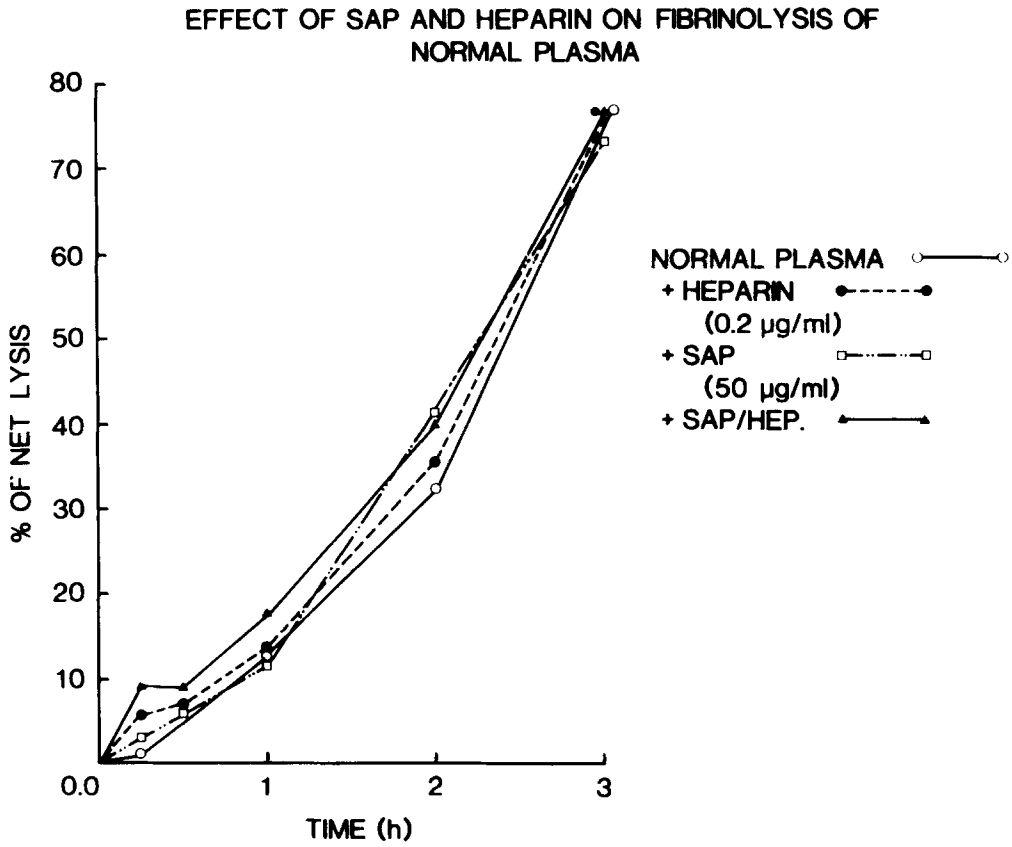


FIGURE 5. Effect of SAP and heparin on fibrinolysis in normal plasma.

TABLE I

*Effects of SAP and Heparin on Thrombin Clot Time, Release of Fibrinopeptide A in Plasma, and Thrombin Activity**

Plasma‡	Clot times	Released FPA	Thrombin activity
	s	*µg/ml*	*U/ml*
Control	23.9	15 (100%)	0.22
+ SAP (50 µg/ml)	26.8	16 (106%)	0.27
+ SAP (200 µg/ml)	56.8	11 (75%)	0.29
+ Heparin (0.8 U/ml)	48.2	9 (60%)	0.17
+ SAP (50 µg/ml) Heparin (0.8 U/ml)	200.0	5 (32%)	0.25

* Clotting assay and FPA were performed with dilute normal pooled plasma, whereas thrombin activity was measured in Tris-NaCl-$CaCl_2$ pH 7.4, using a chromogenic substrate.

‡ Clotting assay and FPA were carried out with 0.1 ml of plasma mixed with 0.4 ml of Tris-NaCl-$CaCl_2$ buffer pH 7.4, or SAP/heparin.

TABLE II
Effect of SAP, Heparin on SAP/Heparin on the Release of Fibrinopeptide A in a Purified System

Fibrinogen*	Clot time	FPA released‡
	s	*μg/ml*
Control	62.9	4.1
+ SAP (80 μg/ml)	58.5	3.9
+ SAP (150 μg/ml)	60.9	4.2
+ Heparin (1 U/ml)	78.0	3.9
+ SAP (80 μg/ml) Heparin (1 U/ml)	98.3	4.4
+ SAP (150 μg/ml) Heparin (1 U/ml)	93.0	4.4

* Fibrinogen was used at 600 μg/ml.
‡ The proteolytic action of thrombin was stopped at 60 s by transferring tubes into ice-water bath.

inhibitor of thrombin in plasma, might play a role in the anticoagulant activity of physiological concentrations of SAP. Indeed, the addition of AT-III and heparin (neither alone) or of hirudin, an exogenous thrombin inhibitor to these component systems, "reconstituted" the substantial anticoagulant synergism between SAP and heparin. These data suggest that the expression of inhibitory effects of physiological concentrations of SAP probably requires the restriction of thrombin activity. As supraphysiological amounts of SAP (200–300 μg/ml) demonstrate an independent ability to inhibit clot formation and FPA release in plasma in the absence of heparin (Table I), these data imply separate but synergistic phenomenon. However, the ability of a low concentration of SAP in the presence of heparin to minimally inhibit clot formation in isolated component systems in the absence of thrombin inhibitors (Table II), indicates the presence of an additional mechanism.

Effects of SAP on the Process of Fibrin Polymerization. The final stage of fibrinogen transformation involves the spontaneous polymerization of fibrin. Upon the release of fibrinopeptide A and B, both end-to-end and lateral associations of fibrin monomers occur to form a noncovalently bonded gel (37). The effect of SAP on fibrin polymerization was evaluated by determining the time required for the transformation of the soluble fibrin monomer into the clot.

The effect of SAP on the process of spontaneous polymerization of fibrin monomer is shown, in part, in Table III, and more completely in Fig. 6. In the control, soluble fibrin monomer polymerized in 365 s (± SD 26s). SAP (25–150 μg/ml) did not interfere with normal fibrin polymerization; heparin (0.5 U/ml) also generated no inhibition of the polymerization process. However, in combination, SAP (50 μg/ml) and heparin provided a substantial synergistic inhibition of polymerization (Fig. 6).

These data illustrate prevention by SAP of spontaneous fibrin polymerization in the presence of heparin, and along with the ability of SAP/heparin to synergistically delay fibrinogen-fibrin conversion, provides a second mechanism by which SAP can participate as an anticoagulant.

TABLE III

Selected Data Illustrating the Effect of SAP and Heparin on the Polymerization of Fibrin

Fibrin monomer	Polymerization time	Range	Increase‡
	s		%
Control	365	335–409	100
+ SAP (50 μg/ml)	369	304–438	101
+ Heparin (0.5 U/ml)	371	316–389	101
+ SAP (50 μg/ml) Heparin (0.5 U/ml)	505	460–578	138
+ Heparin (1 U/ml)	422	416–429	115
+ SAP (50 μg/ml) Heparin (1 U/ml)	536	476–610	147

* SAP and heparin were added to the soluble fibrin monomer before the initiation of polymerization of fibrin at 37°C water-bath.

‡ Control value set at 100%.

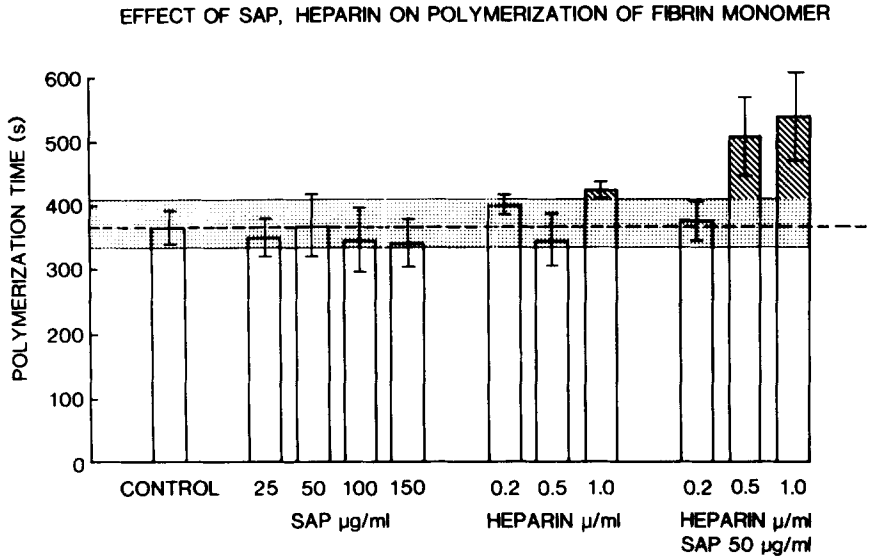


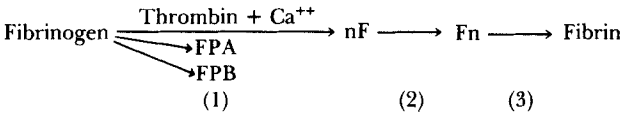
FIGURE 6. Comparison of fibrin monomer polymerization time in the presence or absence of SAP and heparin. The dotted area represents normal ranges of polymerization time, whereas the striped bars represent prolonged polymerization time.

Discussion

SAP at supra-physiological concentrations (150–300 μg/ml) inhibits the coagulation system (Table I and reference 17). The data presented herein also provide evidence that SAP, at physiological concentrations (5–50 μg/ml), markedly decreases the efficacy of both the intrinsic and extrinsic coagulation pathways in the presence of heparin. The inability of SAP to enhance the heparin cofactor activity of antithrombin III shows this not to be the mechanism of increased anticoagulant action. Likewise, the inability of SAP or SAP/heparin to influence Factor XIIIa activity and the presence of enhanced anticoagulant activity in Factor XIII deficient plasmas incubated with SAP/heparin, indicates the absence

of a direct contribution by Factor XIII. In reviewing other reactions that are involved in the final stages of blood coagulation, an altered fibrinolytic system could also result in a delayed onset of fibrin clot formation. To test this possibility, the effect of SAP and heparin on the normal process of fibrinolysis was evaluated. These studies provided no evidence for an influential participation of SAP and/or heparin in fibrinolysis. These data collectively focused our attention on the molecular events involved in the transformation of fibrinogen to fibrin, and the subsequent spontaneous polymerization reaction.

The overall process of fibrinogen transitions can be summarized as follows:



where FPA is fibrinopeptide A, FPB is fibrinopeptide B, nF is fibrin monomer, and Fn is the intermediate polymer.

Thrombin is involved in step *1* to activate fibrinogen; steps *2* and *3* occur spontaneously. The effect of SAP on step *1* was evaluated by monitoring the proteolytic action of thrombin as assessed by the thrombin clot time of plasma, the release of fibrinopeptide A from fibrinogen and the cleavage of p-nitroaniline from a chromogenic substrate. The anticoagulant activity of SAP or SAP/heparin was found to be directly associated with reduction in the generation of fibrinopeptide A. In isolated component systems containing fibrinogen, thrombin, and SAP, the synergistic amplification in the reduction of FPA production was observed only when heparin/anti-thrombin III (not either alone) or hirudin was added to the system. These data suggest that SAP and heparin function independently, but that restricted thrombin activity is essential for physiological concentrations of SAP (5–50 μg/ml) to inhibit blood coagulation. This may explain why supraphysiological concentrations of SAP (150–300 μg/ml) alone inhibit coagulation, i.e., there is a lessened requirement for restricted thrombin activity.

The mechanism(s) by which SAP inhibits FPA release remains unclear, but might reflect an interaction between SAP and fibrinogen that renders fibrinogen resistant to proteolysis. This would delay the onset of fibrin formation since FPA release is essential for the initial end-to-end fibrin aggregation (35). At present, we have two lines of evidence (38) that support this interactive hypothesis. First, ^{125}I-SAP is rapidly incorporated into a forming fibrin clot; second, SAP interacts physically with fibrinogen when measured nephelometrically.

SAP or heparin did not individually interfere with spontaneous fibrin polymerization, whereas, in combination, they synergistically prevented fibrin polymerization. Thus, the ability of SAP and heparin to directly inhibit spontaneous fibrin polymerization provides a second anticoagulative process. The mechanism(s) by which SAP and heparin inhibit fibrin polymerization also remains unclear, but several possibilities exist. First, two fibrin intermediates in the polymerization process have been identified (35, 39), and formation of the fibrin clot can by delayed or prevented by stabilizing these intermediates (40). SAP and heparin, in combination, may interact with the intermediate polymers and stabilize them.

Second, it has been proposed that during spontaneous polymerization the release of fibrinopeptides from the central nodule of fibrinogen allows for intermolecular contact between that central nodule and the terminal nodules of neighboring molecules. Upon release of FPA, newly exposed N-terminals of the α chain serve as the principal site for spontaneous polymerization (41). SAP/heparin may thus prevent spontaneous polymerization by masking these newly exposed N-termini.

Third, the zeta potential may play an important role. The zeta potential is an expression of the electrostatic difference in potentials between the molecular surfaces of the vessel wall, blood elements and plasma proteins, such as fibrinogen and fibrin (42). The human fibrinogen molecule has ~26 more negative charges than positive charges (43). The removal of two pairs of FPA molecules by thrombin reduces the excess negative charges in the central nodule from −8 to −1 (43). When a pair of fibrinopeptide B molecules is released, the central nodule has a +5 net charge (43). Each of the terminal nodules now has a −4 charge (43). This is compatible with each terminal nodule having a complementary electrostatic interaction with a positively charged central nodule during spontaneous polymerization. However, in the presence of SAP and heparin, this complementary electrostatic interaction may be disrupted. Heparin is a highly negatively charged polysaccharide, and is known to alter the zeta potential of fibrinogen and fibrin (42). It is conceivable that SAP and heparin may contribute a large quantity of negative charges to the terminal nodule of fibrin, thus increasing repulsive forces. At the same time, SAP and heparin might neutralize the positive charges in the central nodule of the fibrin monomer.

The potential in vivo relevance of these observations is found in the distribution of SAP and heparin (-like) components in the body. SAP is a normal plasma component in man. Recently a molecule antigenically identical to SAP has been shown to be an integral part of normal vascular basement membranes (15), and to be distributed in the peripheral microfibrillar mantle of elastic fibers in normal skin and blood vessels (16). Heparin has been isolated from various organs and is known to be present in the granules of mast cells and basophils. Free heparin can also be found in the blood circulation of patients under anticoagulant drug therapy. Recently, heparan sulfate, a chemical derivative of heparin, has been found on a variety of cell surfaces including the endothelium (44, 45), and platelets (46). Heparan sulfate has been reported to possess some heparinlike properties including the ability to inhibit blood coagulation (47, 48). Interactions among these substances would likely occur at blood-surface interfaces where many of the enzymes involved in blood coagulation are generated. Thus, SAP and heparin (or heparin-like substances) could be critically placed to delay or prevent fibrin clot formation. By analogy, since SAP and heparin (or heparin-like materials) have been found associated with (or in) vessel walls, their unique anticoagulant activities might present a critical barrier against an activated coagulation state. We suggest that these activities provide a crucial link between thrombo-resistant surface properties and the coagulability of blood.

Summary

Serum amyloid P-component (SAP) is a normal plasma constituent in man with a circulating concentration of ~40 μg/ml. Supraphysiological amounts of SAP (150–300 μg/ml) have been reported to affect coagulation. We have investigated this further by studying the effect of SAP upon clot times in both the absence and presence of heparin, a suggested ligand for SAP and itself a modulator of coagulation processes. In the absence of heparin, SAP (5–125 μg/ml) had no effect on clot times generated by Activated Thrombofax Reagent, brain thromboplastin, Russell's Viper Venom or thrombin when assessed in normal citrated plasma. However, in the presence of amounts of heparin that had only a minor effect upon clot times, SAP (5–40 μg/ml) greatly prolonged clot formation, with the thrombin time the most sensitive to SAP. This suggested that the primary effect of SAP was at this distal level of the coagulation pathway.

Evaluation by radioimmunoassay revealed that supraphysiological concentrations of SAP (150–300 μg/ml) alone reduced by ~25% the release of fibrinopeptide A (FPA) from fibrinogen. In the presence of heparin, substantial synergism was observed with maximal reductions of ~70% in FPA production requiring only 25–50 μg/ml SAP. This inhibition correlated with increased thrombin clot time but was unrelated to any direct modulation in either the activities of anti-thrombin III or activated Factor XIII, and was independent of an alteration in the rate of fibrinolysis. Further, while SAP itself did not interfere with the process of spontaneous fibrin polymerization, in the presence of heparin a prolonged polymerization time (>145%) was observed. We believe that these data reflect the primary mechanisms by which serum amyloid P component influences blood coagulation.

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