Formation, characterization and detection of a ternary complex between S protein, thrombin and antithrombin III in serum

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S protein, a plasma glycoprotein with M_r 78000, has been shown to interfere with the heparin-catalysed inhibition of thrombin by antithrombin III. This interaction was further evaluated in the present study. Native human blood was replaced by either radiolabelled antithrombin III or radiolabelled prothrombin in the reaction mixture, which was incubated at 37 °C. At various time intervals the serum formed from the incubated blood was withdrawn and analysed by crossed immunoelectrophoresis against anti-(S protein) serum in the second dimension. Increasing quantities of radioactivity originating both from antithrombin III and from thrombin were precipitated in a cathodal shoulder to the S protein peak. This observation indicated the formation of a ternary S protein–thrombin–antithrombin III (STAT) complex in serum. This complex could also be observed by the same technique after incubation of purified thrombin in the presence of antithrombin III and S protein. Complex-formation was independent of the presence of heparin and did not require Ca^{2+} ions. Owing to the association of S protein with the thrombin–antithrombin III (TAT) complex, the STAT complex assembled *in vitro* exhibited a higher M_r than the TAT complex as judged by polyacrylamide-gradient-gel electrophoresis in the absence of SDS. Both the serum-originated STAT complex and the STAT complex assembled from purified components sedimented faster than the single components and showed comparable apparent sedimentation coefficients in the range 11–14 S, corresponding to a mean M_r of 350000. The STAT complex could be detected in serum at a dilution of 1:3200 by a sensitive immuno-radiometric assay employing affinity-purified IgG against S protein. These results indicate that S protein, in addition to its role as a heparin-neutralizing factor, becomes incorporated into the nascent TAT complex or can bind to preformed TAT complex during the clotting process.

INTRODUCTION

The plasma proteinase inhibitor antithrombin III inhibits a number of serine proteinases of the coagulation cascade and thereby plays a significant role in the regulation of the haemostatic process (for review see Rosenberg, 1978). Studies conducted with purified proteinase inhibitors provided evidence that antithrombin III is the most effective inhibitor of thrombin in blood (Dowing *et al.*, 1978). The inhibition of thrombin is due to formation of a covalent equimolar enzyme–inhibitor complex, which exhibits an M_r of 90000 under denaturing conditions (Rosenberg & Damus, 1973). Enzyme and inhibitor are linked by an ester bond (Owen *et al.*, 1976), and complex-formation is accompanied by the cleavage at an arginine–serine bond in the antithrombin III molecule (Longas & Finlay, 1980). The thrombin–antithrombin III (TAT) complex contains antigenic structures not present in the parent molecules (Collen & De Cock, 1978), allowing quantification of the complex in plasma (Collen *et al.*, 1977). The low rate at which antithrombin III normally inhibits thrombin is accelerated several-fold by the interaction with heparin (Rosenberg, 1977) or heparin-like structures present at the vessel wall surface (Marcum & Rosenberg, 1984). Heparin induces a conformational change in the inhibitor molecule (Rosenberg & Damus, 1973; Villanueva & Danishefsky, 1977; Nordenman *et al.*,

1978), leading to fast inactivation of thrombin. The heparin-template model for formation of the ternary complex has been shown to fit the kinetic data with respect to heparin–thrombin and heparin–antithrombin III interactions (Griffith, 1982).

The accelerating effect of heparin-like compounds in the initial phase of the inhibition reaction is restricted by the action of neutralizing proteins. Besides albumin, fibrinogen, fibronectin and lipoprotein lipase, platelet factor 4 and histidine-rich glycoprotein have been identified as major heparin-neutralizing proteins (Jordan *et al.*, 1982; Lijnen *et al.*, 1983). Both the last-mentioned proteins neutralize the anticoagulant activity of heparin by interaction with multiple binding sites on the polysaccharide (Lane *et al.*, 1986). In addition, S protein has been described by our laboratory to possess heparin-neutralizing activity by protecting thrombin against fast inactivation by antithrombin III–heparin (Preissner *et al.*, 1985). The heparin-counteracting activity of S protein, which is mainly expressed in the range 0.01–0.1 i.u. of heparin/ml, results in the delay of the fast formation of the covalent complex between enzyme and inhibitor in the initial phase of the inhibition reaction. The interference of S protein in this reaction also leads to an interaction of S protein with the TAT complex, as has been documented by immunoelectrophoresis (Podack & Müller-Eberhard, 1979; Jenne *et al.*, 1985; Preissner & Müller-Berghaus, 1986).

Abbreviations used: TAT complex, thrombin–antithrombin III complex; STAT complex, S protein–thrombin–antithrombin III complex.

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Recently S protein has been demonstrated to be immunologically related to the adhesive protein vitronectin and to promote attachment and spreading of human fibroblasts (Preissner *et al.*, 1986). The amino acid sequence of S protein deduced from the cDNA (Jenne & Stanley, 1985) was shown to be identical with that of vitronectin (Suzuki *et al.*, 1985). An association of vitronectin with the TAT complex in serum has been demonstrated by the use of blotting techniques (Ill & Ruoslahti, 1985).

In the present investigation we obtained evidence that S protein becomes incorporated into a ternary complex with thrombin and antithrombin III during blood coagulation and that the occurrence of high- M_r complexes of TAT in serum is partly due to the formation of this ternary complex.

EXPERIMENTAL

Materials

Bio-Rad protein assay kit and reagents for electrophoresis were obtained from Bio-Rad Laboratories, Munich, West Germany. Analytical-grade sucrose was from Serva, Heidelberg, West Germany. The flexible polyester film Gelbond and agarose were products of Marine Colloids, Rockland, ME, U.S.A. Na¹²⁵I (carrier-free) was obtained from New England Nuclear, Dreieich, West Germany. Heparin (sodium salt) from pig intestinal mucosa, bovine serum albumin, human serum albumin, di-isopropyl phosphorofluoridate and Tween 20 were purchased from Sigma Chemical Co., Munich, West Germany. Polystyrene micro-titre plates (Immuno-plate I F) were obtained from Nunc, Wiesbaden, West Germany. All other chemicals used were analytical grade.

Human S protein was isolated as previously described (Preissner *et al.*, 1985). Established procedures were used to generate and purify human α -thrombin (EC 3.4.21.5) (Morita & Iwanaga, 1981; Lundblad *et al.*, 1975) and to isolate antithrombin III (Miller-Andersson *et al.*, 1974) in a modified version (Preissner *et al.*, 1985). Thrombin had a fibrinogen-clotting activity between 2200 and 2500 N.I.H. units/mg when determined by the method of Fenton & Fasco (1974). All proteins were homogeneous as judged by SDS/polyacrylamide-slab-gel electrophoresis in 10% gels with the buffer system of Laemmli (1970). The isolated proteins were quantified spectrophotometrically by using the following values for $A_{1\%}^{1\text{cm}}$ at 280 nm: S protein, 9.0 (Preissner *et al.*, 1985); antithrombin III, 6.5 (Nordenman *et al.*, 1977); thrombin, 18.3 (Fenton *et al.*, 1977). Antibodies against purified S protein and antithrombin III were raised in rabbits, and the IgG fractions of the antisera were prepared by using standard methods. In addition, affinity-purified IgG against S protein was prepared by passing the whole IgG fraction over a column containing purified S protein coupled to CNBr-activated Sepharose 4B (Pharmacia, Freiburg, West Germany). After adsorption of the antibodies at 4 °C for 24 h and removal of unbound material, S protein-specific IgG was eluted from the column with 0.2 M-glycine/HCl buffer, pH 2.4. Eluted fractions were neutralized by the addition of 2 M-Tris/HCl buffer, pH 8.4. An IgG fraction against human thrombin which does not cross-react with prothrombin was kindly provided by Dr. H. Pelzer, Behringwerke, Marburg, West Germany. Radiolabelling

of proteins was carried out with the Iodogen procedure (Fraker & Speck, 1978) to reach specific radioactivities of 0.5–2 $\mu\text{Ci}/\mu\text{g}$ of protein. Functional properties of the proteins were not affected by the labelling procedure.

Complex-formation between S protein, thrombin and antithrombin III

Ultracentrifugational analysis. A 5 μg portion of thrombin was incubated with 10 μg of radiolabelled antithrombin III and 30 μg of S protein at 37 °C for 20 min. In addition, 300 μl portions of freshly drawn blood supplemented with 1 μCi of ¹²⁵I-antithrombin III/ml in the absence or in the presence of 10 mM-EDTA were incubated at 37 °C for 2 h. After the addition of 2 mM-di-isopropyl phosphorofluoridate, all samples were centrifuged on linear 10–35% (w/v) sucrose density gradients in 20 mM-Tris/HCl buffer, pH 7.4, containing 150 mM-NaCl and 5 mM-EDTA. Ultracentrifugation was carried out at 40000 rev./min in an SW 60 rotor (Beckman Instruments, Fullerton, CA, U.S.A.) for 16 h at 4 °C. After centrifugation, the gradients were fractionated from the bottom of the tubes, and the fractions were analysed for radioactivity. Sedimentation coefficients were approximated by using antithrombin III (3.9 S), IgG (7 S), catalase (12 S) and IgM (19 S) as standards (O'Brien *et al.*, 1978) and are not corrected for temperature and density of the medium.

Electrophoretic and immunochemical methods. Samples (500 μl) of native whole blood were directly withdrawn into plastic tubes containing 0.5 μCi of ¹²⁵I-antithrombin III or 0.5 μCi of ¹²⁵I-prothrombin. The clotting process was stopped after 3 min, 15 min and 120 min by the addition of di-isopropyl phosphorofluoridate (final concentration 2 mM). The control sample contained 10 mM-EDTA and was kept at 37 °C for 120 min. After centrifugation, 15 μl of each supernatant was subjected to crossed immunoelectrophoresis, which was performed by the procedure of Ganrot (1972) on the flexible polyester film Gelbond with 1% (w/v) agarose gel at pH 8.6 in 38 mM-Tris/0.1 M-glycine/2 mM-EDTA. Samples from one experiment were simultaneously run in the first dimension at 5 V/cm for 3 h. The agarose gel in the second dimension contained 4% (v/v) rabbit anti-(human S protein) IgG, and electrophoresis was carried out at 1.5 V/cm for 16 h. After the films had been washed, dried and stained, the distribution of radiolabel was detected by autoradiography.

A similar experiment was performed with purified components: S protein (0.4 mg/ml) was incubated with antithrombin III (0.2 mg/ml) and thrombin (0.04 mg/ml) in the absence and in the presence of 5 i.u. of heparin/ml for 15 min at 37 °C in 20 mM-Tris/HCl buffer, pH 7.4, containing 150 mM-NaCl. The samples, in addition, contained either radiolabelled antithrombin III or radiolabelled thrombin. Analysis by crossed immunoelectrophoresis was performed in the absence or in the presence of 50 i.u. of heparin/ml in the first dimension. Additional experimental steps were identical with the procedure described above.

Complex-formation was also analysed by polyacrylamide-slab-gel electrophoresis in the absence of SDS. The stock solutions of the gradient gel were 2.5% (w/v) and 12.5% (w/v) in acrylamide with an acrylamide/bisacrylamide ratio of 30:0.8, and they contained respectively 2.5% (w/v) and 16% (w/v) sucrose in

90 mM-Tris/80 mM-boric acid/5 mM-EDTA, pH 8.35. For sample preparation, 5 μ g of thrombin was incubated with 10 μ g of radiolabelled antithrombin III at 37 °C for 20 min in the absence or in the presence of 30 μ g of S protein in a total volume of 30 μ l of 20 mM-Tris/HCl buffer, pH 7.4, containing 150 mM-NaCl. Similarly, incubation of thrombin and antithrombin III was carried out in the presence of 15 μ g or 30 μ g of radiolabelled S protein. Samples were subjected to polyacrylamide-gradient-slab-gel electrophoresis at 120 mV/gel for 18 h at 6–8 °C. After the gels had been fixed, stained and dried, the distribution of radiolabel was detected by autoradiography.

Fused rocket immunoelectrophoresis of 20 μ l sucrose-gradient fraction was performed in accordance with Svendsen (1973) in 1% agarose gel in the same buffer as used for crossed immunoelectrophoresis with 2% (v/v) rabbit anti-(human S protein) IgG in the antibody-containing gel. After the films had been washed, dried

and stained, the distribution of radiolabel was detected by autoradiography.

Detection of S protein–thrombin–antithrombin III (STAT) complex by an immuno-radiometric assay

Micro-titration plates were coated with 50 μ l portions of affinity-purified anti-(human thrombin) IgG (7 μ g/ml) at 37 °C for 3 h in phosphate-buffered saline (8 mM-sodium phosphate buffer, pH 7.4, containing 150 mM-NaCl and 0.02% NaN₃). The wells were washed three times with phosphate-buffered saline containing 0.1% Tween 20 and incubated at 22 °C for 1 h with 200 μ l of 3% (w/v) bovine serum albumin dissolved in phosphate-buffered saline. After repetition of the washing procedure the wells were incubated at 22 °C for 2 h with 50 μ l portions of serial dilutions of human EDTA/plasma or serum in phosphate-buffered saline containing 0.1% human serum albumin. After the washing procedure, the wells were incubated with 50 μ l

Fig. 1. Analysis of the formation of the ternary STAT complex in serum by crossed immunoelectrophoresis

Native whole blood was drawn into tubes containing either radiolabelled prothrombin (a) or radiolabelled antithrombin III (b) and kept for 3 min (parts 2), 15 min (parts 3) or 120 min (parts 4) at 37 °C. The control samples (parts 1) were kept at 37 °C for 120 min in the presence of 10 mM-EDTA. Sample supernatants were subjected to crossed immunoelectrophoresis and analysed in the second dimension against anti-(human S protein) IgG. First dimension, anode to the right; second dimension, anode to the top. The upper row in each of (a) and (b) represents the stained gels and the lower row the corresponding autoradiographs.

Fig. 2. Crossed immunoelectrophoresis of the ternary STAT complex assembled from purified components

Thrombin, antithrombin III and S protein were incubated in molar proportions of 1:3:5 either in the presence of radiolabelled antithrombin III (a and b) or radiolabelled thrombin (c and d) and subjected to crossed immunoelectrophoresis in the absence (a and c) or in the presence (b and d) of heparin in the first dimension (anode to the right). Electrophoresis in the second dimension (anode to the top) was carried out in agarose gel containing anti-(human S protein) IgG. Autoradiographs of the gels are shown.

of radiolabelled affinity-purified anti-(human S protein) IgG (8 μ Ci/ml) at 22 °C for 3 h. After an additional washing step, the wells were counted for radioactivity. Duplicate measurements were performed, and data were corrected for the non-specific antibody binding that was observed in those wells that contained buffer only. In parallel, serial dilutions of purified S protein were employed and assayed by the same procedure.

RESULTS

Formation of STAT complex

The formation of ternary complex consisting of S protein, thrombin and antithrombin III in clotting blood was demonstrated by crossed immunoelectrophoresis in a time-course reaction (Fig. 1). Blood not exposed to anticoagulant was supplemented with either radiolabelled prothrombin (Fig. 1a) or antithrombin III (Fig. 1b) and allowed to clot for various time intervals. Incorporation of thrombin as well as antithrombin III together with S protein in a complex was evidenced by precipitation of radioactivity originating from (pro)-thrombin or antithrombin III by anti-(S protein) IgG. Both radiolabelled immunoprecipitates formed were found at the same position, located cathodal to the original S protein peak in anticoagulated plasma (Fig. 1, parts 3 and 4). During serum formation the incorporation of increasing amounts of radiolabel into a ternary complex was noted and was maximal after 2 h. In contrast, 2 h incubation of native blood anticoagulated with EDTA did not reveal any precipitation of prothrombin or antithrombin III alone by anti-(S protein) IgG (Fig. 1, parts 1).

In a purified system, the three components thrombin,

antithrombin III and S protein were incubated in molar proportions 1:3:5 either in the presence or in the absence of heparin. Subsequent formation of the ternary complex was documented by crossed immunoelectrophoresis (Fig. 2). Inspection of the autoradiographs revealed the precipitation of both radiolabelled antithrombin III (Figs. 2a and 2b) and thrombin (Figs. 2c and 2d) by anti-(S protein) IgG at identical positions, indicating the incorporation of S protein into a ternary complex with thrombin and antithrombin III. Complex-formation itself and the electrophoretic mobility of the complex were independent of the presence of heparin or Ca^{2+} ions. In control experiments anti-(S protein) IgG did not precipitate any radiolabel from ^{125}I -antithrombin III/S protein or ^{125}I -thrombin/S protein mixtures. Similarly, anti-(antithrombin III) IgG precipitated radiolabelled S protein only from mixtures consisting of all three components but not from ^{125}I -S protein/antithrombin III samples (results not shown).

Formation of the ternary complex in serum or in an isolated system with thrombin, antithrombin III and S protein was further analysed by ultracentrifugation in linear sucrose density gradients (Fig. 3). In this series of experiments radiolabelled antithrombin III was used as a marker. Whereas antithrombin III in EDTA/plasma sedimented as a symmetrical peak at a position of 3.9 S, a faster-migrating shoulder was apparent in serum. When purified components were used for complex-formation, two distinct peaks became separated during ultracentrifugation: the slower-migrating peak corresponded to antithrombin III in plasma, whereas the faster-migrating peak was found in the same range as the shoulder of the antithrombin III peak in serum. In order to verify complex-formation, the gradient fractions were analysed by fused rocket immunoelectrophoresis against anti-(S protein) IgG. No radioactivity representing antithrombin III was precipitated by the antibody in the EDTA/plasma sample. In serum and in the purified system, however, complex-formation was confirmed by the appearance of radiolabelled immunoprecipitates (Fig. 3a, parts B and C, arrows). In both gradients the ternary complex appeared at corresponding positions with sedimentation coefficients of 11–14 S, well separated from the majority of plasma proteins or uncomplexed components.

The high- M_r nature of the ternary complex consisting of S protein, thrombin and antithrombin III (STAT) was further approached by using polyacrylamide-gradient-gel electrophoresis in the absence of SDS (Fig. 4). The use of a polyacrylamide-gradient gel allowed the separation of proteins or complexes of proteins according to their apparent size. Charge differences were mostly abolished by using a pH of 8.35 in the buffer system. Relative to the positions of radiolabelled antithrombin III (Fig. 4, lane a) and S protein (Fig. 4, lane b), reaction of thrombin with ^{125}I -antithrombin III alone (Fig. 4, lane c) facilitated the formation of the major broad band(s) representing the thrombin–antithrombin III (TAT) complex. This band migrated more slowly into the gradient gel than did antithrombin III alone. Incubation of thrombin with ^{125}I -antithrombin III in the presence of S protein, however, resulted in the appearance of even slower-migrating bands (Fig. 4, lane d). When S protein instead of antithrombin III contained the radiolabel, a complex with identical mobility and band pattern was detectable (Fig. 4, lanes e and f).

Fig. 3. Ultracentrifugation of the ternary STAT complex through linear sucrose density gradients

The distribution of radiolabelled antithrombin III in EDTA/plasma (parts A), serum (parts B) and the STAT complex assembled from purified components (parts C) was followed by ultracentrifugation; sedimentation direction is to the left. (a) Upper panels, fused rocket analysis of gradient fractions against anti-(human S protein) IgG; lower panels, corresponding autoradiographs of the stained gels. (b) Radioactivity profiles of gradient fractions. Note the appearance of additional radiolabelled immunoprecipitates (arrows) in parts B and C in (a), which correspond to the occurrence of high- M_r material in the gradient profiles.

Quantification of the STAT complex in serum by an immuno-radiometric assay

In an immuno-radiometric assay anti-(human thrombin) IgG fixed to the micro-titre plate was applied to recognize the STAT complex in serum. A radiolabelled affinity-purified antibody against S protein was subsequently employed to quantify bound STAT complex in serial dilutions of serum. A typical result of STAT detection by the immuno-radiometric assay is depicted in Fig. 5. Although the binding of radiolabelled antibody to dilutions of serum and plasma revealed a dose-response curve in both cases, binding to serum samples was much more pronounced. Since dilutions of purified S protein at the same concentrations as in dilutions of plasma gave a similar antibody-binding response (results not shown), adsorption of S protein in plasma samples accounted for background values in the assay. Subtraction of these values from those in serum of the same dilution resulted in a dose-response curve (Fig. 5, ● symbols), which exhibited saturation at higher serum concentrations. At a dilution of serum of 1:3200 STAT complex was still detectable.

DISCUSSION

S protein was initially detected as inhibitor of the assembly of the membrane-attack complex of comple-

ment (Kolb & Müller-Eberhard, 1975; Podack *et al.*, 1984) and has been shown to be identical with vitronectin (Jenne *et al.*, 1985; Preissner *et al.*, 1986). As a unique multifunctional protein it thereby serves as an adhesive protein and spreading factor (Barnes & Silnutzer, 1983; Hayman *et al.*, 1983) and heparin-neutralizing protein (Preissner & Müller-Berghaus, 1986). The present investigation provides evidence for the additional role of S protein as component in a ternary complex together with thrombin and antithrombin III (STAT complex). This complex was formed during the clotting process in blood and was detectable by immunoelectrophoretic techniques as well as a sensitive immuno-radiometric assay in serum. Consequently, whenever inhibition of thrombin by antithrombin III occurs during blood coagulation, the occurrence of this ternary complex will have to be taken into consideration.

Complex-formation was independent of the presence of Ca^{2+} or heparin, and the complex appeared to be heterogeneous with respect to molecular size and exhibits a mean sedimentation coefficient (s) of 12.5 S. By employing the sedimentation coefficient of 4.6 S for isolated S protein, a mean apparent M_r of 350000 is calculated according to:

$$s_{(\text{STAT})/s_{(\text{S protein})}} = (M_{r(\text{STAT})}/M_{r(\text{S protein})})^{\frac{2}{3}}$$

Fig. 4. Polyacrylamide-gradient-gel electrophoresis of TAT and STAT complexes in the absence of SDS

Radiolabelled antithrombin III (lane a), radiolabelled S protein (lane b), TAT complex containing ^{125}I -antithrombin III (lane c), STAT complex containing ^{125}I -antithrombin III (lane d) and STAT complex containing ^{125}I -S protein (lanes e and f) were electrophoresed on the same gel for 18 h at 6–8 °C. The anode is at the bottom. Autoradiographs of the gels are shown.

(Martin & Ames, 1961), suggesting an equimolar distribution of components within the complex in the molar proportions 2:2:2. Polyacrylamide-gradient-gel electrophoresis of the STAT complex in comparison with the TAT complex revealed an increase in M_r to about 200 000–400 000, due to the association of S protein with TAT. Since S protein contains a free thiol group (Dahlbäck & Podack, 1985; Preissner *et al.*, 1985), the appearance of two high- M_r bands (Fig. 4) may indicate the participation of monomeric as well as dimeric S protein in complex-formation.

High- M_r complexes containing thrombin and antithrombin III with M_r 200 000 have already been documented in serum by gel filtration (Binder, 1973; Pepper *et al.*, 1977; Marciniak & Gora-Maslak, 1983). The appearance of these complexes has been proposed to be due to aggregation of the TAT complex. In light of our results, however, the binding of S protein to the nascent TAT complex is at least partly responsible for the formation of such high- M_r complexes in serum. Although binding of S protein to antithrombin III or prothrombin was not observed and no covalent linkage

Fig. 5. Immuno-radiometric assay of STAT complex in serum

In parallel, dilutions of normal human plasma and normal human serum were incubated with anti-(human thrombin) IgG, adsorbed on micro-titration wells. Subsequent incubation with radiolabelled affinity-purified anti-(human S protein) IgG was used to quantify the STAT complex. The dose-response curve (●) was obtained by subtraction of values for plasma (background) from those for serum at the same sample dilution.

between S protein and the TAT complex was evident (Preissner & Müller-Berghaus, 1986), it is suggested that S protein may bind to both, nascent as well as pre-formed TAT complex. However, the mechanism of interaction of thrombin, antithrombin III and S protein within the STAT complex remains unclear.

Since S protein participates in the inhibition reaction of thrombin by antithrombin III, S protein has become an integral component in the regulation of the activity of the enzyme in the circulation. This has already been documented for the initial phase of the heparin-catalysed inhibition of thrombin by antithrombin III, in which S protein protects the enzyme against fast inactivation (Preissner *et al.*, 1985; Preissner & Müller-Berghaus, 1986). In addition, the association of S protein with the TAT complex in the terminal stage of the inhibition reaction will endow the ternary STAT complex with yet unrecognized properties. Owing to the cell-attachment site in S protein (Suzuki *et al.*, 1984), binding of STAT complex to cell surfaces will be promoted, and thus a rapid disappearance of the complex from the blood-stream may be expected. The reported rapid clearance rate of exogenously administered TAT complex from the circulation in animals (Shifman & Pizzo, 1982) may confirm the association of S protein with the TAT complex *in vivo*. Experiments performed *in vitro* that documented the specific binding of the TAT complex to hepatocytes (Bauer *et al.*, 1982; Fuchs *et al.*, 1984; Fair & Plow, 1986) may have to be re-evaluated in order to

elucidate the interaction of these cells with the 'true' ligand *in vivo*, the STAT complex. Yet another consequence for the terminal stage of coagulation *in vivo* appears to be the association of adhesive properties of S protein with mitogenic properties of thrombin (Chen & Buchanan, 1975) within the STAT complex, facilitating increased cell attachment and subsequent cell growth at the site of cell injury.

As S protein fulfils an additional role as inhibitor of the terminal assembly for the membrane-attack complex of complement by preventing lysis of innocent bystander cells (McLeod *et al.*, 1974), complement activation of serum will result in the formation of the hydrophilic macromolecular SC5b-9 complex (Kolb & Müller-Eberhard, 1975), in which S protein is associated with the terminal complement components. Since in serum a fraction of S protein is bound within the ternary STAT complex, one may expect that subsequent complement activation of serum will lead to the incorporation of free as well as TAT-associated S protein within the SC5b-9 complex. This conclusion may explain the finding that antithrombin III is indeed associated with SC5b-9 in complement-activated serum (Kolb & Kolb, 1983); however, the mechanism of formation of the SC5b-9 complex *in vivo* remains to be established.

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REFERENCES

- Barnes, D. W. & Silnutzer, J. (1983) *J. Biol. Chem.* **258**, 12548–12552
- Bauer, P. I., Mandl, J., Machovich, R., Antoni, F., Garzo, T. & Horvath, I. (1982) *Thromb. Res.* **28**, 595–606
- Binder, B. (1973) *Thromb. Diath. Haemorrh.* **30**, 280–283
- Chen, L. B. & Buchanan, J. M. (1975) *Proc. Natl. Acad. Sci. U.S.A.* **72**, 131–135
- Collen, D. & De Cock, F. (1978) *Biochim. Biophys. Acta* **525**, 287–290
- Collen, D., De Cock, F. & Verstraete, M. (1977) *Eur. J. Clin. Invest.* **7**, 407–411
- Dahlbäck, B. & Podack, E. R. (1985) *Biochemistry* **24**, 2368–2374
- Dowing, M. R., Bloom, J. W. & Mann, K. G. (1978) *Biochemistry* **17**, 2649–2653
- Fair, D. S. & Plow, E. F. (1986) *Thromb. Res.* **41**, 67–78
- Fenton, J. W. & Fasco, M. J. (1974) *Thromb. Res.* **4**, 809–817
- Fenton, J. W., Fasco, M. J., Stackrow, A. B., Aronson, D. L., Young, A. & Finlayson, J. S. (1977) *J. Biol. Chem.* **252**, 3587–3598
- Fraker, P. J. & Speck, J. C. (1978) *Biochem. Biophys. Res. Commun.* **80**, 849–857
- Fuchs, H. E., Shifman, M. A., Michalopoulos, G. & Pizzo, S. V. (1984) *J. Cell. Biochem.* **24**, 197–206
- Ganrot, P. O. (1972) *Scand. J. Clin. Lab. Invest.* **29**, Suppl. **124**, 39–47
- Griffith, M. J. (1982) *J. Biol. Chem.* **257**, 7360–7365
- Hayman, E. G., Pierschbacher, M. D., Öhgren, Y. & Ruoslahti, E. (1983) *Proc. Natl. Acad. Sci. U.S.A.* **80**, 4003–4007
- Ill, C. R. & Ruoslahti, E. (1985) *J. Biol. Chem.* **260**, 15610–15615
- Jenne, D. & Stanley, K. K. (1985) *EMBO J.* **4**, 3153–3157
- Jenne, D., Hugo, F. & Bhakdi, S. (1985) *Thromb. Res.* **38**, 401–412
- Jordan, R. E., Favreau, L. V., Braswell, E. H. & Rosenberg, R. D. (1982) *J. Biol. Chem.* **257**, 400–406
- Kolb, W. P. & Kolb, L. M. (1983) *Biochemistry* **22**, 496–504
- Kolb, W. P. & Müller-Eberhard, H. J. (1975) *J. Exp. Med.* **141**, 724–735
- Laemmli, U. K. (1970) *Nature (London)* **227**, 680–685
- Lane, D. A., Pejler, G., Flynn, A. M., Thompson, E. A. & Lindahl, U. (1986) *J. Biol. Chem.* **261**, 3980–3986
- Lijnen, H. R., Hoylaerts, M. & Collen, D. (1983) *J. Biol. Chem.* **258**, 3803–3808
- Longas, M. A. & Finlay, T. H. (1980) *Biochem. J.* **189**, 481–489
- Lundblad, R. L., Uhteg, L. C., Vogel, C. N., Kingdon, H. S. & Mann, K. G. (1975) *Biochem. Biophys. Res. Commun.* **66**, 482–489
- Marciniak, E. & Gora-Maslak, G. (1983) *Thromb. Haemostasis* **49**, 32–36
- Marcum, J. A. & Rosenberg, R. D. (1984) *Biochemistry* **23**, 1730–1737
- Martin, R. G. & Ames, B. N. (1961) *J. Biol. Chem.* **236**, 1372–1379
- McLeod, B. C., Baker, P. & Gewurz, H. (1974) *Immunology* **26**, 1145–1157
- Miller-Andersson, M., Borg, H. & Andersson, L.-O. (1974) *Thromb. Res.* **5**, 439–452
- Morita, T. & Iwanaga, S. (1981) *Methods Enzymol.* **6**, 819–831
- Nordenman, B., Nyström, C. & Björk, I. (1977) *Eur. J. Biochem.* **78**, 195–203
- Nordenman, B., Danielsson, A. & Björk, I. (1978) *Eur. J. Biochem.* **90**, 1–6
- O'Brien, R. D., Timpone, C. A. & Gibson, R. E. (1978) *Anal. Biochem.* **86**, 602–615
- Owen, W. G., Penick, G. D., Yoder, E. & Poole, B. L. (1976) *Thromb. Haemostasis* **35**, 87–95
- Pepper, D. S., Banhegyi, D. & Cash, J. D. (1977) *Thromb. Haemostasis* **38**, 494–503
- Podack, E. R. & Müller-Eberhard, H. J. (1979) *J. Biol. Chem.* **254**, 9908–9914
- Podack, E. R., Preissner, K. T. & Müller-Eberhard, H. J. (1984) *Acta Pathol. Microbiol. Immunol. Scand. Sect. C* **92**, Suppl. **284**, 89–96
- Preissner, K. T. & Müller-Berghaus, G. (1986) *Eur. J. Biochem.* **156**, 645–650
- Preissner, K. T., Wassmuth, R. & Müller-Berghaus, G. (1985) *Biochem. J.* **231**, 349–355
- Preissner, K. T., Heimbürger, N., Anders, E. & Müller-Berghaus, G. (1986) *Biochem. Biophys. Res. Commun.* **134**, 951–956
- Rosenberg, R. D. (1977) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **36**, 10–18
- Rosenberg, R. D. (1978) *Annu. Rev. Med.* **29**, 367–378
- Rosenberg, R. D. & Damus, P. S. (1973) *J. Biol. Chem.* **248**, 6490–6505
- Shifman, M. A. & Pizzo, S. V. (1982) *J. Biol. Chem.* **257**, 3243–3248
- Suzuki, S., Pierschbacher, M. D., Hayman, E. G., Nguyen, K., Öhgren, Y. & Ruoslahti, E. (1984) *J. Biol. Chem.* **259**, 15307–15314
- Suzuki, S., Oldberg, A., Hayman, E. G., Pierschbacher, M. D. & Ruoslahti, E. (1985) *EMBO J.* **4**, 2519–2524
- Svendsen, P. J. (1973) in *A Manual of Quantitative Immuno-electrophoresis* (Axelson, N. H., Krøll, J. & Weeke, B., eds.), pp. 69–70, Universitetsforlaget, Oslo
- Villanueva, G. B. & Danishefsky, I. (1977) *Biochem. Biophys. Res. Commun.* **74**, 803–809