

INTERACTION OF SOLUBLE FIBROBLAST SURFACE
ANTIGEN WITH FIBRINOGEN AND FIBRIN

Identity with Cold Insoluble Globulin of Human Plasma*

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A cell-type-specific membrane glycoprotein (SFA) of chicken (1) and human (2) fibroblasts has been recently described from our laboratory. Malignantly transformed fibroblasts lack SFA on their surface (3). The same antigen is present in serum and its molecular properties are similar to those of the cellular antigen (4). The surface labeled protein present on normal but not on transformed hamster fibroblasts (5, 6) seems to be the hamster counterpart of SFA.¹ The serum concentration of SFA has been estimated to be in the order of 100 $\mu\text{g/ml}$ and it was originally thought to represent a previously unknown serum protein.

Here we show that SFA interacts with fibrin and appears in the serum cryoglobulin fraction associated with cryofibrinogen. SFA has properties similar to those of "cold insoluble globulin" (CIG) (7-9) and the two proteins seem to be identical.

Materials and Methods

Reagents. Bovine fibrinogen (80% clottable) and fibrin powder were kindly provided by Dr. Gunnar Myllylä (The Finnish Red Cross Blood Transfusion Center, Helsinki). Human fibrinogen (90% clottable) was obtained from Kabi (Sweden) and was further purified as described by Finlayson and Mosesson (10). The purified preparation did not reveal any proteins other than fibrinogen when tested in immunodiffusion at the concentration of 1 mg/ml against antinormal human serum (Behringwerke, Marburg). No SFA, a major contaminant of the original preparation, was detectable in the purified fibrinogen.

Antisera. Production of antisera to SFA has been described (2). Human fibroblasts (MRC-5) cultured in 10% fetal calf serum were washed with buffer and treated with insoluble papain for 15 min. The material released was dialyzed, lyophilized, and used for immunization of rabbits or sheep. Such antisera were absorbed with calf serum and insolubilized papain and reacted with a single component in fibroblast extracts and human serum or plasma in immunodiffusion.

Matrix-Bound Proteins. Fibrinogen was linked to Sepharose beads (Pharmacia Fine Chemicals, Inc., Sweden) according to Porath et al. (11).

Purification of CIG. Outdated human plasma taken to citrate was used as starting material and CIG was purified by cryoprecipitation followed by glycine precipitation and DEAE cellulose chromatography as described by Mosesson and Umfleet (9).

Affinity Chromatography on Sepharose-Conjugated Purified Human Fibrinogen. The Sepharose

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¹ Keski-Oja, J., A. Vaehri, and E. Ruoslahti. 1974. Fibroblast surface antigen (SF): the external glycoprotein lost in proteolytic stimulation and malignant transformation. Manuscript submitted for publication.

beads, 1.5 ml, were washed with 8 M urea and suspended in phosphate-buffered saline (PBS) (5 ml) containing 10^{-3} M-phenyl methyl sulphonyl fluoride (PMSF) as protease inhibitor. Human serum (1 ml) was added and the mixture was shaken overnight at 0°C. The particles were washed with ice-cold PBS (10 ml) three times and subsequently eluted by transferring the beads to +37°C. Three eluates with PBS (5 ml), each incubated for 5 min at +37°C, were collected. The eluates were concentrated and analyzed for protein by the method of Lowry et al. (12) and for SFA by radial immunodiffusion (13). Other human serum proteins were detected using immunodiffusion against antinormal human serum.

Results

Interaction of SFA with Fibrin and Fibrinogen. The ambient temperature during the formation of the blood coagulation clot and its removal by centrifugation greatly influenced the concentration of SFA in the resulting serum sample (Table I). Serum prepared at +37°C contained slightly less SFA than the corresponding plasma samples processed at +37°C, whereas serum obtained at 0°C had about 20% of this concentration. The original SFA concentration was partly restored to samples clotted at 0°C if they were transferred to +37°C and centrifuged to remove the clot at this temperature. No further removal of SFA after removal of the clot could be achieved by centrifugation at low temperature.

The above results suggested that SFA was bound to the fibrin clot at low temperature and that this binding could be prevented or reversed at +37°C. Such binding was directly proven by subsequent experiments. SFA from serum was bound to Sepharose-conjugated bovine or human fibrinogen (Fig. 1) and to powdered human fibrin at 0°C and released at +37°C. No binding of SFA could be demonstrated to plain Sepharose particles or particles to which a human serum albumin fraction was conjugated. Of the SFA present in the original serum sample 70-80% was bound to fibrinogen-Sepharose and about 5% could be recovered showing a 10-fold purification of SFA relative to other proteins.

Co-purification of SFA and CIG. The purification procedure of CIG according to Mosesson and Umfleet (9) showed concentration of SFA in the cryoprecipitate fraction of human plasma (7- to 10-fold relative to other serum proteins) in two experiments. The final product contained SFA with some fibrinogen and small amounts of other serum proteins as analyzed by immunodiffusion. A reaction of immunological identity was obtained with cellular SFA obtained with papain digestion and with SFA from fibrinogen affinity chromatography.

TABLE I
SFA Concentration in Samples of Plasma and Serum Prepared in Two Experiments Using Various Temperatures

Sample	Temperature		SFA concentration (% of plasma level)	
	During clotting	During removal of clot or cells by centrifugation	Exp. 1	Exp. 2
Plasma	—	37°C	100	100
Serum	37°C	37°C	100	95
Serum	0°C	+4°C	10	35
Serum	0°C	37°C	—	95

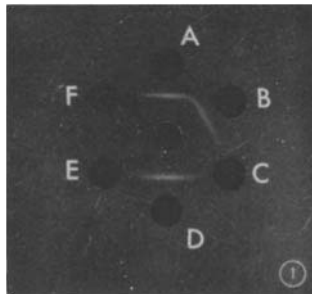


FIG. 1. Immunodiffusion in agarose. Normal human serum A and D. Material bound to and released from: B, Sepharose-conjugated fibrinogen; C, unconjugated Sepharose; D, Sepharose-conjugated albumin fraction; and E, material obtained from a control tube without Sepharose. Center well, anti-SFA.

Discussion

Co-purification of the fibroblast SF antigen present in serum and the cold insoluble globulin CIG indicates that CIG and the serum SFA are the same protein. This notion is reinforced by their similar S values. These are 12.3 S for CIG (9) and 13.5 S for SFA (2). Our recent results show that the polypeptide composition of human SFA (unpublished) closely resembles that of chicken SFA (4) in being composed of two chains with mol wt close to 210,000 and a minor component with a mol wt of about 150,000. This is in agreement with the reported composition of CIG of two chains with mol wt of about 250,000 (14).

SFA is present on the surface of fibroblasts (1, 2) and glial cells (unpublished observations) but not on several other types of cells. It is shed from the surface of *in vitro* cultured fibroblasts and a parallel phenomenon occurring *in vivo* may account for its presence in serum and plasma. It therefore seems that the protein previously known as CIG originates from fibroblasts.

CIG is regularly found, along with cryofibrinogen, in the cryoprecipitate fraction of human plasma, hence the name cold insoluble globulin (7). This may not be an appropriate designation for this protein, since our results show that SFA/CIG is not insoluble at low temperatures as such. Centrifugation of serum at 0°C failed to alter the SFA concentration of serum. It is likely that the association of SFA with the plasma cryoprecipitate fraction is due to its affinity to cryofibrinogen rather than its insolubility at low temperature. Our experiments show that such an interaction does occur. SFA was bound to insolubilized fibrinogen and fibrin at 0°C and could be released by elevating the temperature. This phenomenon could be utilized to achieve a significant degree of purification (10-fold) in a single exceedingly simple step.

The biological significance of the affinity of SFA to fibrin and fibrinogen is a matter of speculation. Our unpublished data suggest that the cellular form of SFA binds actin and we have evidence indicating that SFA is located in fibrillar structures of the cell surface probably associated with microfilaments (15). The affinity of soluble SFA towards fibrinogen and fibrin could be demonstrated at low temperature only. However, since the molecular mechanism of this interaction is not known and results such as the requirement of high temperature for

dissociation of the complex suggest involvement of enzymatic events, it is conceivable that interaction of cell surface SFA molecules with fibrin(ogen) may have physiological significance.

Fibroblasts transformed with tumor viruses lack SFA (3, footnote 2). Such cells show increased fibrinolytic activity in vitro (16, 17). The characteristics of transformed fibroblasts attributable to the increased fibrinolytic activity are pronounced when the cells are plated on fibrin layer (18). Whether interaction of the fibroblast surface SFA with fibrin plays any role in these phenomena remains to be seen.

Summary

A cell-type specific glycoprotein antigen (SFA) from fibroblast surface appears in human plasma and serum. The amount of SFA in serum was reduced if the blood coagulation clot was removed at a low temperature. SFA could be bound to Sepharose-conjugated fibrinogen and to fibrin powder at 0°C and was subsequently released when the temperature was elevated to +37°C. This procedure resulted in a 10-fold enrichment of SFA relative to other serum proteins.

SFA was found to be concentrated in the cryoprecipitate fraction of human plasma and was copurified with the cold insoluble globulin (CIG) with procedures published for the purification of the latter component. SFA/CIG is not insoluble at low temperatures as such and its appearance in the cryoprecipitate fraction of plasma is likely to be due to its affinity to cryofibrinogen evident from these experiments. The biological significance of the interaction of fibroblast surface SFA molecules with fibrin(ogen) is not known.

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