ON COUPLING BOVINE FIBRINOGEN TO THE SURFACE OF MALIGNANT MURINE PLASMA CELLS BY MEANS OF TRANSGLUTAMINASE

L. Fésüs* and K. Laki

National Institute of Arthritis, Metabolism and Digestive Diseases
National Institutes of Health, Public Health Service
U. S. Department of Health, Education and Welfare
Bethesda, Maryland 20014

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SUMMARY: It was found that native, as well as ^{125}I labelled fibrinogen, may be coupled by means of transglutaminase to surface proteins of malignant plasma cells of the mouse. Binding of fibrinogen, although greatly affecting the [^{14}C] putrescine labelling of several surface proteins, leaves the cells viable and malignant.

INTRODUCTION

It is well established now that plasma transglutaminase (activated Factor XIII) is intimately involved in the proliferation of granulation tissue during wound healing (1-3).

According to one explanation, fibroblasts can proliferate properly only on fibrin cross-bonded by plasma transglutaminase. Another explanation is offered by Mosher who, in a recent paper (4), proposes that plasma transglutaminase promotes the proliferation of fibroblasts by coupling fibrin to a protein on the membrane of the fibroblasts. He bases this proposition on his finding that plasma transglutaminase cross-bonds insoluble globulin of blood plasma to fibrin, and on the work of Rouslahti and Vaheri (5), who identify cold insoluble globulin with a protein component of the fibroblast membrane.

In a series of papers, Laki and co-workers (6-10) found evidence that tissue transglutaminase is involved in the proliferation of tumor tissues. Because of a certain similarity of tumor proliferation to the proliferation

*Visiting Fellow. Permanent Address:
Institute of Pathophysiology, Medical School
University of Debrecen, H-4012 Hungary

of granulation tissue, we explore in this paper whether, by using tissue transglutaminase, fibrinogen could be coupled to some membrane protein, as Mosher proposes, on the surface of the malignant plasma cell of the mouse.

The experiments reported here show that fibrinogen can be attached to membrane proteins and that the modified cells remain viable.

MATERIALS AND METHODS

Lactoperoxidase (Calbiochem); Carrier-free ^{125}I (New England Nuclear); Bolton-Hunter reagent (New England Nuclear); bovine fibrinogen (Armour Pharmaceutical) purified further by the method of Laki (11) and labelled by the lactoperoxidase technique (12) or the Bolton-Hunter reagent (13); materials for the SDS* polyacrylamide gel electrophoresis (Canalco); [^{14}C] putrescine-dihydrochloride (New England Nuclear; 51.8 mCi per mM); NCS** solubilizer (Amersham); aquasol scintillation fluid (New England Nuclear); guinea pig liver transglutaminase (spec. activity 7.12; kind gift of R. Chung, NIDR-NIH).

Tumor cells were obtained from the peritoneal cavity of YPC-1 plasma cell tumor-bearing mice (CAF_1) on the 12-14th day after the i.p. passage. Following the removal of red cells by lowering the ionic strength to 0.05M for 30 seconds, the tumor cells were washed three times in cold Tris-saline solution (pH 7.4) containing 139 mM NaCl, 2.7 mM KCl, 5.6 mM glucose, 25 mM Tris HCl, 5 mM CaCl and 0.5 mM EDTA. The cells were handled in plastic tubes and their viability was checked by trypan blue staining throughout the experiments.

Binding studies with labelled fibrinogen: 50 µg per ml solutions (in Tris-saline of the ^{125}I labelled fibrinogens were incubated at 37°C in the presence or absence of 25 µg transglutaminase for 25 min. (first incubation). Afterwards, an excess amount of EDTA, (final concentration 3mM), was added to the transglutaminase-containing tubes and half of that to the others. A portion of each solution was denatured in the presence of 1% SDS and 1% 2-mercaptoethanol. To the remaining part of the solutions, washed tumor cells were mixed to obtain a cell suspension containing 10^6 cells per ml. Into those tubes to which neither transglutaminase nor excess EDTA had been previously added, 25 µg of transglutaminase was mixed. Following 25 min. incubation at 37°C (second incubation), the cells were washed 3x with 1:20 volume of cold Tris-saline. The radioactivity of the pellets were counted either directly or they (300-400 µg of protein) were made 1% in SDS and 1% in 2-mercaptoethanol, treated at 90°C for 5 min., submitted to SDS gel electrophoresis in 5% polyacrylamide disc gels according to the method of Weber and Osborn (14) parallel with those samples denatured earlier, and the radioactivity of the 1 mm thick pieces of the fixed and sliced gels were counted by a Beckman γ-counter.

Surface labelling of original and modified tumor cells: Washed tumor cells (10^6 per ml in Tris-saline) were incubated with or without 50 µg fibrinogen and 25 µg transglutaminase per ml for 25 min. at 37°C. The incubation was followed by washing 3 x with cold Tris-saline, then by labelling the surface proteins by means of transglutaminase catalyzed binding of [^{14}C] putrescine into them. The reaction mixture contained

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* Sodium dodecyl sulfate

** NCS solubilizer is a solution of a quaternary ammonium base in toluene

the tumor cells (10^6 per ml) suspended in Tris-saline, dithiothreitol (5mM), [^{14}C] putrescine (1mM), and transglutaminase (50 µg per ml). Following 30 min. incubation, the cells were washed 3x1:20 volume of cold Tris-saline and the pellets were prepared for SDS gel electrophoresis as described above. Molecular weight marker proteins (cytochrome C, rabbit skeletal muscle actin, α, β and γ chains of bovine fibrinogen, bovine serum albumin and human γ-globulin) were co-electrophoresed with these samples for the estimation of the molecular weights of the tumor cell surface proteins labelled by this technique. The gels were stained or sliced into 1 mm thick pieces after fixation, and their radioactivity was determined by scintillation counting.

RESULTS

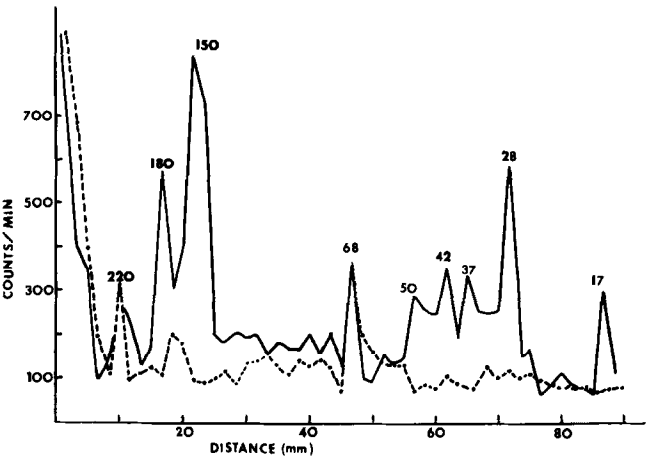
The results of the experiments are summarized in Table I and Figure 1. It is seen from Table I that labelling fibrinogen either by the lactoperoxidase iodination (12) of tyrosine residues, (spec. activity: 15 µCi per mg), or by the ^{125}I labelled acylating agent (13), which reacts with the free amino groups (spec. activity: 10.3 µCi per mg), the three chains of the molecules could be iodinated effectively, the β chain taking up most of the label (Table I, A). There is some attachment of labelled fibrinogen to the cells, but in this non-specific binding, the fibrinogen molecules remain unchanged (Table I, B). Cross-linking of the labelled fibrinogen by transglutaminase before mixing them with the tumor cells resulted in characteristic patterns of the distribution of radioactivity on the gel using both labelling techniques (Table I, C). When cross-linked fibrinogen was incubated with the tumor cells, the number of fibrinogen molecules (calculated on the basis of the original specific activities) bound to the cells increased slightly (more in the case of fibrinogen labelled by the Bolton-Hunter reagent), but the molecular composition of the cell-attached cross-linked fibrinogen remained the same as that without the addition of the cells (Table I, D). On the other hand, the number of bound fibrinogen molecules increased greatly when transglutaminase operated in the presence of the cells and fibrinogen (Table I, E). In this case, the patterns of the distribution of fibrinogen-related radioactivity on the gels were totally different from those observed under conditions A, B, C and D in Table I due to transglutaminase-catalyzed binding. Irrespective whether fibrinogen was labelled by lactoperoxidase or by the Bolton-Hunter

TABLE I

The number of cell-bound fibrinogen molecules and the distribution of the fibrinogen-related radioactivity on SDS gels following electrophoresis

EXPERIMENTAL CONDITIONS		X 10^6 FIBRINOGEN MOLECULES PER CELL	% DISTRIBUTION OF RADIOACTIVITY ON THE GELS					
			Not penetrated into gel	Polymers	γ - γ dimer	Chains of fibrinogen α	β	γ
A	1					14.5 ± 2.4	55.3 ± 4.8	30.2 ± 2.8
	2					32.4 ± 3.6	46.2 ± 3.5	20.4 ± 2.9
B	1	0.8 ± 0.18				15.2 ± 2.2	55.5 ± 4.7	29.3 ± 3.1
	2	0.9 ± 0.15				31.9 ± 3.5	47.5 ± 3.6	20.6 ± 2.9
C	1		2.1 ± 0.4	17.4 ± 1.9	2.9 ± 0.9	8.3 ± 1.2	46.8 ± 3.6	22.5 ± 2.0
	2		6.0 ± 1.2	51.1 ± 4.8	3.0 ± 0.4	3.8 ± 0.9	26.2 ± 1.8	9.9 ± 0.9
D	1	1.0 ± 0.15	4.2 ± 0.5	16.6 ± 2.0	1.6 ± 0.7	8.1 ± 1.4	47.5 ± 3.2	22.0 ± 2.2
	2	1.2 ± 0.05	9.3 ± 1.3	49.0 ± 5.1	2.3 ± 0.3	3.1 ± 0.8	26.9 ± 1.5	9.4 ± 1.0
E	1	2.5 ± 0.32	78.2 ± 6.0	11.0 ± 2.9	1.3 ± 0.4	1.1 ± 0.2	5.4 ± 0.3	3.0 ± 0.2
	2	2.4 ± 0.28	80.1 ± 6.3	14.2 ± 3.0	0.8 ± 0.1	1.0 ± 0.2	2.7 ± 0.3	1.2 ± 0.1

Experimental conditions: A. Fibrinogen-incubation-EDTA; B. Fibrinogen-incubation-EDTA-cells-incubation; C. Fibrinogen+transglutaminase-incubation-EDTA; D. Fibrinogen+transglutaminase-incubation-EDTA-cells-incubation; E. Fibrinogen-incubation cells+transglutaminase-incubation. Fibrinogen was labelled by the lactoperoxidase technique (1) or the Bolton-Hunter reagent (2).
Mean±SE of 5 experiments in each case.



Legend to Fig. 1

Gel electrophoresis pattern of surface-labelled proteins of the original (solid curve) and the modified (broken line) tumor cells using the transglutaminase-catalyzed incorporation of [^{14}C] putrescine for their labelling. The numbers at the peaks when multiplied by 10^3 refer to apparent molecular weights.

reagent most of the radioactivity applied onto the gels did not penetrate, suggesting the formation of high molecular weight polymers of membrane proteins and fibrinogen. Apparently all three chains of fibrinogen provide sites for transglutaminase to connect them to surface proteins. Increasing the fibrinogen concentration in the cell suspension, the transglutaminase-catalyzed binding into the surface membrane could be elevated up to 10^7 molecules per cell as a maximum in our experimental conditions.

In order to gain an idea of what surface proteins may be involved in the transglutaminase-catalyzed finding of fibrinogen, we first labelled the surface proteins with [^{14}C] putrescine and transglutaminase. It is seen from Fig. 1 that nine definite proteins of the tumor cell surface could be labelled by the transglutaminase-catalyzed incorporation of radioactive putrescine (Fig. 1). The same pattern was found when fibrinogen, either cross-linked or not, was added to the cell suspension without transglutaminase

(not included in Fig. 1). When fibrinogen was incorporated into the surface prior to the putrescine labelling (broken curve, Fig. 1), most of these surface proteins became unavailable for putrescine incorporation.

The YPC-1 malignant plasma cells implanted intraperitoneally into CAF_1 mice after transglutaminase-catalyzed attachment of fibrinogen into their surface proteins killed the animals in two weeks as did the untreated cells. This experiment demonstrates that this kind of modification of the surface properties did not affect the malignancy of the cells.

DISCUSSION

In this paper we describe two types of experiments: (1) a coupling of labelled fibrinogen to membrane proteins of the malignant plasma cell of the mouse; (2) showing that after coupling non-labelled fibrinogen to surface proteins, the labelling pattern of these surface proteins changes.

Under the conditions described in Methods, about 10^6 fibrinogen molecules become incorporated into the surface of a cell.

After coupling fibrinogen into the surface, many surface proteins lose their ability to incorporate putrescine. Fibrinogen may attach to the surface proteins at a site that otherwise would take up putrescine, or fibrinogen binding to a particular protein may cover others and prevent transglutaminase from catalyzing the attachment of putrescine to these proteins. At present, we cannot choose between these two possibilities.

The proteins rendered inaccessible to labelling after fibrinogen incorporation (Fig. 1) have a range of molecular weights from 17,000 up to 280,000. The small quantity of 220,000 molecular weight protein is in line with the observation, that this surface protein diminishes on malignant transformation. In view of Mosher's (4) proposition it is surprising that it does not react with fibrinogen. An other protein reacting with fibrinogen has a MW of about 42,000 in the range of the major histocompatibility antigen (H-2). It will be interesting to see whether these surfaces modified cells can escape the histocompatibility barrier.

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