Binding and Degradation of Platelet Thrombospondin by Cultured Fibroblasts

PAULA J. McKEOWN-LONGO, ROXANN HANNING, and DEANE F. MOSHER
Department of Medicine, University of Wisconsin, Madison, Wisconsin 53706

ABSTRACT Thrombospondin was purified from human platelets and labeled with $^{125}$I, and its metabolism was quantified in cell cultures of human embryonic lung fibroblasts. $^{125}$I-Thrombospondin bound to the cell layer. The binding reached an apparent steady state within 45 min. Trichloroacetic acid-soluble radioactivity was detected in the medium after 30 min of incubation; the rate of degradation of $^{125}$I-thrombospondin was linear for several hours thereafter. Degradation of $^{125}$I-thrombospondin was saturable. The apparent $K_m$ and $V_{max}$ for degradation at $37^\circ$C were $6 \times 10^{-11}$ M and $1.4 \times 10^5$ molecules per cell per minute, respectively. Degradation was inhibited by chloroquine or by lowering the temperature to $4^\circ$C. Experiments in which cultures were incubated with thrombospondin for 45 min and then incubated in medium containing no thrombospondin revealed two fractions of bound thrombospondin. One fraction was localized by indirect immunofluorescence to punctate structures; these structures were lost coincident with the rapid degradation of 50–80% of bound $^{125}$I-thrombospondin. The second fraction was localized to a trypsin-sensitive, fibrillar, extracellular matrix. $^{125}$I-Thrombospondin in the matrix was slowly degraded over a period of hours. Binding of $^{125}$I-thrombospondin to the extracellular matrix was not saturable and indeed was enhanced at thrombospondin concentrations $>3 \times 10^{-8}$ M. The ability of $^{125}$I-thrombospondin to bind to extracellular matrix was diminished tenfold by limited proteolytic cleavage with trypsin. Degradation of trypsinized $^{125}$I-thrombospondin was also diminished, although to a lesser extent than matrix binding. Heparin inhibited both degradation and matrix binding. These results suggest that thrombospondin may play a transitory role in matrix formation and/or organization and that specific receptors on the cell surface are responsible for the selective removal of thrombospondin from the extracellular fluid and matrix.

MATERIALS AND METHODS

Materials: Ham's nutrient medium (F-12) and Hanks' balanced salt solution were obtained from Grand Island Biological Co. (Grand Island, NY). Fetal calf serum was from Sterile Systems (Logan, UT). Bio-Gel P-300, heparin-
agarose and electrophoresis materials were from Bio-Rad Laboratories (Richmond, CA). Na\textsuperscript{125}I was from New England Nuclear (Boston, MA). Fluorescein-conjugated rabbit anti-mouse IgG was from Cappel Laboratories (Cooperville, PA). N-Ethylmaleimide was from Pierce Chemical Co. (Rockford, IL). Materials for autoradiography were from Kodak (Rochester, NY). Heparin and other biochemicals were from Sigma Chemical Co. (St. Louis, MO). Human plasma fibronectin was purified from a by-product of Factor VIII production (15). Type I collagen from fetal bovine skin (16) and fibronogen (17) and \alpha2-macroglobulin (18) from human plasma were purified according to published procedures.

**Cell Culture:** Human embryonic lung fibroblasts were derived from a locally established strain (Dr. Catherine Reznikoff, University of Wisconsin). Cells were grown in F-12 nutrient medium containing 10% fetal calf serum, 100 U/ml penicillin, 50 \mu g/ml streptomycin and 2 \mu g/ml fungazole. Cells were customarily split 1:10 every 7 d, and experiments were done on cells between passages 4 and 15. All experiments were done using confluent cell layers in 60-mm tissue culture plates. At confluence, cultures contained ~5 x 10\textsuperscript{5} cells, as determined after trypsinization by enumeration in a hemocytometer.

**Preparation of Isolated Matrices:** Isolated cell matrices were prepared from confluent embryonic lung fibroblast cultures by extraction with 1% deoxycholate in 20 mM Tris-HCl buffer (pH 8.3) containing 2 mM phenylmethylsulfonylfluoride, 2 mM ethylene diamine tetraacetic acid, 2 mM N-ethylmaleimide, and 2 mM iodoacetic acid. Extractions were carried out for 10 min at room temperature. Matrices were rinsed three times with Hank's balanced salt solution before use.

**Purification and iodination of Thrombospondin:** Thrombospondin was purified from thrombin-activated human platelets by gel filtration chromatography on Bio-Gel P-300 and affinity chromatography on heparin-agarose as described previously (8). Thrombospondin concentration was calculated on the basis of a published extinction coefficient (3). Purified thrombospondin, ~ 200 \mu g, was iodinated with 1 mCi Na\textsuperscript{125}I by the chloramine-T method (19). The iodinated protein was repurified on heparin-agarose and electrophoresed on 8% polyacrylamide gels (data not shown). When 125I-thrombospondin (lanes c and d) was reduced with 2-mercaptoethanol before electrophoresis on an 8% polyacrylamide gel, there were minor bands of 145,000, 103,000, and 80,000 mol wt which were seen in reduced but not unreduced thrombospondin (in Fig. 1, compare lane c to lane d).

**Immunofluorescence:** For indirect immunofluorescent staining, splats were dried and autoradiographed with XAR-2 film.

**Gel Electrophoresis:** SDS PAGE was performed on slabs of 8% separating and 3.3% stacking gels using a discontinuous buffer system (20). Marker proteins (see reference 8) were visualized by staining with Coomassie Brilliant Blue. For visualizing 125I-thrombospondin, slabs were dried and autoradiographed with XAR-2 film.

**RESULTS**

**Time Course of Binding and Turnover of Thrombospondin by Fibroblast Cell Cultures**

Confluent cell layers of human embryonic lung fibroblasts were incubated with 125I-thrombospondin for 4 h, and binding and degradation of thrombospondin by the cell layers were quantified (Fig. 2). Thrombospondin became rapidly associated with the cell layer, and maximum binding was achieved within 45 min. Degradation of thrombospondin by the cell layers was first detected at 30 min and proceeded at a linear rate between 45 and 150 min. The amount of thrombospondin degraded after 4 h represented 12% of the added radioactivity. The remaining 88% was precipitable in trichloracetic acid and migrated as intact thrombospondin in polyacrylamide gels (data not shown). When 125I-thrombospondin was allowed to bind to cell layers for 45 min and then "chased" (Fig. 2, arrow), the bound fraction had two fates. After 3 h of "chase," 60% of the material bound at 45 min had been with fluorescein-conjugated rabbit anti-mouse IgG diluted 1:100 in Hanks' buffered salt solution. Coverslips were put onto glass slides in Tris-buffered saline, pH 7.4, containing 1 mg/ml phenylenediamine. Photography was performed with a Leitz microscope equipped with epifluorescence and phase contrast.
FIGURE 2 Time course of binding and degradation of thrombospondin by confluent human embryonic lung fibroblasts. Cultures were incubated with 3 ml of binding medium containing 1 μg/ml thrombospondin (300,000 cpm/ml) for the designated times. Cell layers were either incubated continuously with 125I-thrombospondin (●, ●) or incubated with 125I-thrombospondin for 45 min and then "chased" (arrow) with fresh binding medium containing no thrombospondin (○, ○). Degradation was monitored by the appearance of trichloroacetic acid-soluble radioactivity (△, △) in the medium. Cell layers were solubilized in sodium hydroxide to determine bound thrombospondin (●, ●).

degraded. The rate of degradation decreased during the "chase" period, and the remaining 125I-thrombospondin remained bound in the cell layer. Bound 125I-thrombospondin was judged to be intact thrombospondin when analyzed by SDS PAGE (data not shown). Intermediate degradation products of thrombospondin were never detected, either in the medium or in the cell layer, indicating that thrombospondin was rapidly degraded into small fragments.

Immunofluorescent Localization of Bound Thrombospondin

To investigate further the two fractions of cell-associated thrombospondin, we incubated cell layers with purified platelet thrombospondin, and thrombospondin was then localized by indirect immunofluorescence using a mouse monoclonal antibody prepared against platelet thrombospondin. The monoclonal antibody recognizes human thrombospondin synthesized by cultured cells and the bovine thrombospondin present in fetal calf serum (10). Therefore, cell layers were incubated with two concentrations of purified human platelet thrombospondin, either 1 or 20 μg/ml. The increased fluorescent staining seen in cultures labeled with 20 μg/ml thrombospondin was assumed to represent the exogenous thrombospondin added during the experiment. Cultures were incubated with thrombospondin for 45 min. At 45 min, one set of cultures was processed for immunofluorescence, and the other set was "chased" for 3 h. Cultures incubated for 45 min showed both a punctate and a fibrillar type fluorescence when fixed with paraformaldehyde and acetone (Fig. 3, a and c). This pattern was similar to that detected when cell layers grown in fetal calf serum are analyzed by immunofluorescence with antithrombospondin (9, 11). Punctate fluorescence was much less prominent in cells fixed only with paraformaldehyde (data not shown), suggesting that some of the punctate structures were intracellular. "Chased" cell layers exhibited fibrillar fluorescence patterns (Fig. 3, b and d). The fibrillar fluorescence patterns were similar in cell layers fixed only in paraformaldehyde, indicating that these fibrils were extracellular (data not shown). These data in combination with Fig. 2 suggest that the punctate fluorescence represents thrombospondin in endocytic vesicles destined for degradation in lysosomes. These structures were not present after a 3-h "chase" and therefore do not represent synthetic pools. Rather, the diffuse perinuclear staining pointed out by arrows in Fig. 3, c and d probably represents recently synthesized thrombospondin in the rough endoplasmic reticulum and Golgi complex.

Effect of Concentration on Degradation and Matrix Binding of Thrombospondin

Cell layers were incubated with increasing concentrations of 125I-thrombospondin, and both the rate of thrombospondin degradation and the amount of thrombospondin bound in the matrix were determined (Fig. 4a). The rate of thrombospondin degradation approached saturation at concentrations >20 μg/ml. Thrombospondin binding in the extracellular matrix, however, did not saturate over the concentration range studied. Instead, the proportion of thrombospondin bound into the matrix increased with increasing thrombospondin concentrations, suggesting that deposition of throm-
The binding and degradation of thrombospondin by fibroblast cell layers was studied. Confluent cell layers were incubated with 2 ml of binding medium containing increasing concentrations of a mixture of $^{125}$I-thrombospondin and unlabeled thrombospondin. The specific activity of this mixture was 4 $\mu$Ci/mg. Degradation rates of $^{125}$I-thrombospondin (---) were determined between 45 and 180 min of incubation. Fordetermination of bound thrombospondin (O-O), cultures were incubated with $^{125}$I-thrombospondin for 45 min, "chased" for 3 h in unlabeled medium, and solubilized in 1 M sodium hydroxide. Binding (O-O) and degradation (---) on blank plates were also monitored. The turnover data were replotted by the Lineweaver-Burke method for calculation of kinetic constants (b).

Inhibition of Degradation and Matrix Binding of Thrombospondin

Inasmuch as various amines have been shown to block receptor-mediated protein degradation, probably by raising the pH of endocytic vesicles (21) or lysosomes (22), the effects of such amines on thrombospondin metabolism were tested. Chloroquine at $>1 \times 10^{-4}$ M completely blocked thrombospondin degradation (Fig. 5). In similar experiments, both methylyamine and ammonium chloride were also effective inhibitors of thrombospondin turnover at concentrations of $1 \times 10^{-2}$ M (data not shown).

Degradation of $^{125}$I-thrombospondin was inhibited by low temperature, i.e., there was no detectable increase in trichloroacetic acid-soluble radioactivity in the medium of cultures incubated at 4°C even after 4 h of incubation (data not shown). The ability of fragments of trypsinized thrombospondin to be bound and degraded by fibroblasts was also tested (Table I). Mild trypsinization of $^{125}$I-thrombospondin resulted in two labeled fragments, one having a nonreduced molecular weight of ~300,000 and a second of <30,000 which moved at the dye front (Fig. 1). The large fragment was composed of disulfide-bonded subunits with individual sizes of 90,000 mol wt. This fragment has been designated the tryptic-resistant core of thrombospondin (2, 3, 23). The <30,000-mol-wt

### Table 1

| % Total trichloroacetic acid precipitable cpm added | Degraded | Bound | After 180-min chase |
|-----------------------------------------------|----------|-------|---------------------|
| Control $^{125}$I-thrombospondin              | 5.7      | 5.1   | 1.7                 |
| "Mock-trypsinized" $^{125}$I-thrombospondin   | 6.1      | 5.5   | 1.5                 |
| Trypsinized $^{125}$I-thrombospondin          | 4.0      | 2.1   | 0.17                |

$^{125}$I-Thrombospondin (400,000 cpm) was treated with trypsin (2 $\mu$g/ml in Tris-buffered saline), inactive or "mock" trypsin (2 $\mu$g/ml 1 mg/ml soybean trypsin inhibitor), or Tris-buffered saline alone for 10 min at 37°C. Soybean trypsin inhibitor, 20 $\mu$g/ml, was then added to trypsin-treated and control incubations. $^{125}$I-Thrombospondin in binding medium (100,000 cpm/ml) was incubated with cell layers. Degradation was measured between 45 and 135 min of incubation as appearance of trichloroacetic acid-soluble radioactivity in the medium. For determination of binding, cell layers were labeled for 45 min and then scraped directly into 1 M sodium hydroxide or incubated for 180 min in binding medium containing no thrombospondin before solubilization in sodium hydroxide. Determinations represent the average from duplicate plates which did not differ by >3%.
labeled matrices and confluent cell cultures were incubated with 125I-thrombospondin for up to 4 h. 125I-Thrombospondin was degraded in intact cultures (Fig. 6a) but not by isolated matrices (Fig. 6b). Initial binding to the matrix proceeded at a slower rate than to intact cultures, and binding was linear over 4 h. Matrices that were "chased" after 45 min (Fig. 6b, arrow) lost little thrombospondin over the subsequent 3 h. That exogenous thrombospondin was bound to the matrix in deoxycholate-extracted cell layers was demonstrated by indirect immunofluorescent localization of thrombospondin to matrix fibrils (data not shown).

Thrombospondin turnover in the matrices of intact cell layers and cell-free matrices was also compared over a longer timespan (Fig. 7). In intact cell layers, 75% of bound 125I-thrombospondin was lost in a biphasic manner from the cell layer over a period of 44 h. Loss of 125I-thrombospondin from the cell layers was accompanied by a corresponding increase in trichloroacetic acid–soluble radioactivity in the culture medium. In contrast, only 20% of bound thrombospondin was lost from the isolated matrices after 44 h. None of the radioactivity in culture medium of isolated matrices became soluble in trichloroacetic acid, suggesting that the small proportion of intact thrombospondin that was lost from the matrices was lost by diffusion rather than by degradation. Degradation of matrix-bound thrombospondin in intact cell layers was inhibited by chloroquine, even in the presence of medium conditioned by cells not treated with chloroquine (data not shown). This finding suggests that the turnover of matrix-bound thrombospondin occurs intracellularly and not by the action of a secreted protease.

125I-Thrombospondin bound to the matrix of intact cell layers was treated with trypsin and various extractants (Table III). Of the agents tested, trypsin (88% release) and 3 M guanidine hydrochloride (64% release) were the most effective. Although heparin blocked thrombospondin incorporation into the matrix (Table II), heparin, 100 μg/ml, extracted

**Table II**

| Macromolecule   | Degradation (%) of control | Matrix Incorporation |
|-----------------|-----------------------------|----------------------|
| Collagen        | 92                          | 118                  |
| 25 μg/ml        | 95                          | 106                  |
| 125 μg/ml       | 100                         | 96                   |
| a2-Macroglobulin| 25 μg/ml                    | 94                   |
| 125 μg/ml       | 94                          | 102                  |
| Heparin         | 27                          | 12                   |
| 5 μg/ml         | 30                          | 10                   |

Cell layers were incubated with 125I-thrombospondin (1 μg/ml; 250,000 cpm/ml) in binding medium containing the above macromolecules at the indicated concentrations. Degradation was measured as trichloroacetic acid–soluble radioactivity appearing in the medium between 55 and 180 min of incubation. Matrix incorporation was measured as the amount of radioactivity remaining in the cell layer after labeling cultures for 45 min and "chasing" for 3 h in binding medium containing no thrombospondin. The data are expressed as percent of control levels, and determinations represent averages of duplicates that varied by <5%.

![Figure 6](image-url)  
**Figure 6** Thrombospondin binding and degradation by isolated cell matrices. 125I-Thrombospondin (5 μg/ml, 400,000 cpm/ml) was incubated with intact (a) and 1% deoxycholate-extracted (b) fibroblast cell layers. Cultures were either incubated continuously with 125I-thrombospondin (○) or incubated with 125I-thrombospondin for 45 min and then "chased" (arrow) for 3 h with fresh binding medium containing no thrombospondin (○). Background binding (30–120 ng) and degradation (1–20 ng) on blank plates have been subtracted from each time point.
little thrombospondin from the matrix when compared to Hanks' buffered salt solution alone. Sodium chloride, 1 M, and 1% deoxycholate were moderately effective extractants.

**Discussion**

The present experiments were modeled after our recent investigations of fibronectin (28). Like thrombospondin, fibronectin is synthesized by cells in culture and incorporated into the extracellular matrix (9, 10). In addition, plasma fibronectin in serum-containing growth medium becomes incorporated into the extracellular matrix of the cultured cells (28, 29). Plasma fibronectin also becomes localized in connective tissues in vivo (30). As described in the introduction, thrombospondin synthesized by cultured cells and platelet thrombospondin are very similar if not identical. Thrombospondin is present in plasma in only trace amounts (31, 32), and concentrations of exogenous thrombospondin in vivo are probably significant only in localized wound areas where there is dermalization of platelets. Fetal calf serum contains 30–40 µg/ml thrombospondin as assayed by our enzyme-linked immunoabsorbent assay (10; unpublished results). Therefore, the usual conditions of cell culture mimic a wound area in the sense that exogenous thrombospondin is present in serum-containing growth medium.

Using 125I-thrombospondin purified from human platelets, we demonstrated that exogenous thrombospondin does bind to cell layers of cultured human embryonic lung fibroblasts. A portion of the bound thrombospondin was degraded to a form that is soluble in trichloroacetic acid. The remainder formed a more stable association with the cell layer. This stable fraction of thrombospondin could be localized to the extracellular matrix (Fig. 3) where it was present in fibrillar structures reminiscent of fibrils known to contain fibronectin, collagen, and proteoglycans (13). We also prepared thrombospondin in the presence of calcium to maintain calcium-sensitive structures within the molecule (33). Thrombospondin prepared in this manner was bound and degraded by cultured fibroblasts with kinetics similar to those shown in Fig. 2.

Immunofluorescent localization of thrombospondin in punctate structures after 45 min and the subsequent disappearance of thrombospondin in these structures after a 3-h “chase” (Fig. 3) coincident with the rapid appearance of trichloroacetic acid-soluble radioactivity in the medium (Fig. 2) suggest that the punctate fluorescence seen in cells stained with antithrombospondin (9, 10) represents thrombospondin in coated pits, endocytic vesicles, and lysosomes. Degradation of thrombospondin was a saturable process (Fig. 4a) that could be inhibited by weak bases (Fig. 5) and low temperature (4°C). Rapid turnover of thrombospondin, therefore, is very similar to the specific and saturable receptor-mediated endocytosis and degradation of α2-macroglobulin (24–26) and low density lipoprotein (34). The fact that trypsinized fragments of thrombospondin were degraded less rapidly than intact thrombospondin (Table I) also indicates that turnover occurs by specific endocytosis rather than a more generalized process, i.e., fluid phase pinocytosis (35). Degradation of thrombospondin (Table II) and low density lipoprotein (36) is inhibited by heparin. Both proteins bind to heparin (23, 37). In the case of low density lipoprotein, heparin may compete with the cell surface receptor for ligand binding (36). The same may be true for thrombospondin.

Binding of thrombospondin to the extracellular matrix was not saturable. Indeed, increasing concentrations of thrombospondin resulted in higher proportions of thrombospondin remaining in the matrix (Fig. 4a). Thus, interactions among molecules at higher concentrations may alter the way in which thrombospondin associates with the matrix, and exogenous thrombospondin concentration may regulate the content of matrix-bound thrombospondin. Thrombospondin bound directly to isolated cell matrices prepared by deoxycholate extraction (Fig. 6b). Plasma fibronectin, in contrast, does not bind directly to the extracellular matrix, but to a cellular receptor that apparently mediates its assembly into the matrix (28).

Full release of thrombospondin from the matrix required proteolysis or strong denaturation (Table III), suggesting that thrombospondin is tightly bound to the matrix. Similar ex-
traction methods must be employed to remove 125I-plasma fibronectin from the matrix (28), and unpublished observations). Matrix-bound thrombospondin was slowly degraded and, after 24 h, 60% of it was in a trichloroacetic acid-soluble form (Fig. 7). In contrast, there is no degradation of matrix-bound 125I-fibronectin over the same time period (28).

Thrombospondin has been reported to interact with fibronectin and collagen when one of the molecules is adsorbed onto plastic (5, 14). However, collagen and fibronectin did not have significant effects on thrombospondin binding or degradation by fibroblast cell layers. The inhibition of thrombospondin binding to matrix by heparin suggests a possible role for heparan sulfate proteoglycans in the metabolism of thrombospondin by these cell cultures. Heparan sulfate proteoglycans are present in the extracellular matrix (13) and may be integral components of the plasma membrane (38, 39). Heparin and a concentration of sodium chloride considerably higher than that required to disrupt heparin-thrombospondin binding (3), however, were only slightly effective in releasing thrombospondin from the matrix. Thus, further studies are needed to sort out the interactions responsible for initial and long-term binding of thrombospondin to the matrix.

This work was supported by grants from the National Institutes of Health (NIH), HL 21644 and HL 29586. P. J. McKeown-Longo was supported by NIH F 32 HL 06652 and NIH T32 HD 07118. D. F. Mosher was an Established Investigator of the American Heart Association and its Wisconsin Affiliate.

Received for publication 10 June 1983, and in revised form 26 September 1983.

REFERENCES

1. Baezinger, N. L., G. N. Brodie, and P. W. Majerus. 1972. Isolation and properties of a thrombin-sensitive protein of human platelets. J. Biol. Chem. 247:2727-2731.
2. Lawler, J. W., H. S. Slattery, and J. E. Colligan. 1978. Isolation and characterization of platelet thrombospondin. J. Biol. Chem. 253:8609-8616.
3. Margossian, S. S., J. W. Lawler, and H. S. Slattery. 1981. Physical characterization of platelet thrombospondin. J. Biol. Chem. 256:7495-7500.
4. Jaffe, E. A., L. K. Leung, L. R. Nachman, R. I. Levin, and D. F. Mosher. 1982. Thrombospondin is the endogenous lectin of human platelets. Nature (London) 295:236-248.
5. Lahav, J., M. A. Schwartz, and R. O. Hynes. 1982. Analysis of platelet adherence with a radioactive chemical crosslinking reagent: interaction of thrombospondin with fibronectin and collagen. Cell 31:253-262.
6. McPherson, J. H., S. Sage, and P. Bornstein. 1981. Isolation and characterization of a glycoprotein secreted by aortic endothelial cells in culture: apparent identity with platelet thrombospondin. J. Biol. Chem. 256:11330-11336.
7. Sage, H., P. Prizio, and P. Bornstein. 1981. Secretory phenotypes of endothelial cells in culture: comparison of aortic, venous, capillary, and corneal endothelium. Annu. Rev. Cell Biol. 1:427-442.
8. Mosher, D. F., M. J. Doyle, and J. A. Jaffe. 1982. Synthesis and secretion of thrombospondin by cultured human endothelial cells. J. Cell Biol. 93:343-348.
9. Raugi, G. J., S. M. Murray, D. Abbas-Brown, and P. Bornstein. 1983. Thrombospondin: synthesis and secretion by cells in culture. J. Cell Biol. 95:351-354.
10. Jaffe, E. A., J. T. Ruggiero, L. K. Leung, M. J. Doyle, P. J. McKeown-Longo, and D. F. Mosher. 1983. Culture of human fibroblasts to synthesize and secrete thrombospondin and incorporate it into extracellular matrix. Proc. Natl. Acad. Sci. USA. 80:998-1002.
11. Carter, W. G., and S. Hakomori. 1981. A new cell surface antigen-detected insoluble glycoprotein matrix of human and hamster fibroblast. J. Biol. Chem. 256:6950-6953.
12. Chen, L. B., A. Murray, A. B. Segal, and M. L. Wahl. 1978. Studies on intercellular L3T4 glycoprotein matrices. Cell. 14:377-391.
13. Hedman, K., S. Johansson, T. Vartio, L. Kujala, and A. Vahteri. 1982. Structure of the pericellular matrix of hamster chondrocytes with ganglioside-GM1. J. Cell Biol. 95:465-472.
14. Lawler, J. W., and H. S. Slattery. 1981. The role of heparin binding peptides from platelet thrombospondin by proteolytic action of thrombin, plasmin and trypsin. Proc. Natl. Acad. Sci. USA. 78:267-279.
15. Mosher, D. F., and A. Vahteri. 1980. Binding and degradation by asialo-globulin by cultured fibroblasts. Biochem. Biophys. Acta. 627:113-122.
16. Van Leenen, F., J. J. C. van der Merwe, and A. van Deurs. 1978. Uptake and degradation of asialo-globulin by cultured fibroblasts. Exp. Cell Res. 112:273-282.
17. Dickson, R. B., M. C. Wittingham, and I. Pasta. 1981. Binding and internalization of 125I-asialo-globulin by cultured fibroblasts. J. Biol. Chem. 256:3454-3459.
18. Hedman, K., J. Kurkiniemi, K. Altsto, A. Vahteri, S. Johansson, and M. Hicok. 1979. Evidence for calcium-sensitive structure in pericellular matrix: association of heparan and chondroitin sulphates with asialo-globulin. Proc. Natl. Acad. Sci. USA. 76:3218-3221.
19. Saglio, S. D., and H. S. Slattery. 1982. The use of a radionuclide assay to quantify thrombospondin. Blood. 59:162-166.
20. Mosher, D. F., E. C. Williams, and P. J. McKeown-Longo. 1983. Metabolism of thrombospondin and fibronectin by endothelial cells in culture. In The Biology of Endothelial Cells. E. A. Jaffe, editor. Martinus Nijhoff, The Hague. In press.
21. Lawler, J. W., J. C. Choo, and C. M. Cohen. 1982. Evidence for calcium-sensitive structure in platelet thrombospondin: isolation and partial characterization of thrombospondin in the presence of calcium. J. Biol. Chem. 257:12257-12265.
22. Goldstein, J. L., and M. S. Brown. 1974. Evidence and quantitation of low density lipoproteins by cultured human fibroblasts. J. Biol. Chem. 249:5135-5162.
23. Steinman, R. M., I. S. Melman, W. A. Muller, and Z. A. Cohn. 1983. Endocytosis and recycling of plasma membrane. J. Cell Biol. 96:1-27.
24. Goldstein, J. L., S. K. Basu, G. Y. Brunshed, and M. S. Brown. 1976. Release of low density lipoprotein from its cell surface receptor by sulfated glycosaminoglycans. Cell. 7:85-95.
25. Goldstein, J. L., I. P. Petersson, and M. Hicok. 1981. Cell surface heparan sulfate: an intercalated membrane glycoprotein. Proc. Natl. Acad. Sci. USA. 78:5371-5375.
26. Rapprager, A. C., and M. Bernfield. 1983. Heparan sulfate proteoglycan from mouse lymphoid cell membranes. J. Biol. Chem. 258:3632-3636.