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Engvall E & Ruoslahti E. Binding of soluble form of fibroblast surface protein, fibronectin, to collagen. *Int. J. Cancer* 20:1-5, 1977.
[Division of Immunology, City of Hope National Medical Center, Duarte, CA]

The paper shows that fibronectin binds to collagen, in particular to denatured collagen, or gelatin, and that fibronectin can be purified using affinity chromatography on gelatin-Sepharose. [The *SCI*® indicates that this paper has been cited in over 1,315 publications.]

Interactions with Fibronectins

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This work was started soon after Erkki Ruoslahti and I had moved to the City of Hope Medical Center in Duarte, California, from the University of Helsinki, Finland. Erkki was one of the discoverers of "SF antigen" (surface-of fibroblast antigen), later renamed "fibronectin." I was a postdoc. One of my projects was to study possible interactions of fibronectin with other proteins. It was already known that fibronectin bound weakly to fibrinogen and fibrin[1] and that it was present in connective tissue and extracellular matrices, possibly because it was bound to another extracellular protein. I was using a modified ELISA[2] to study the interaction of fibronectin with other proteins, and the assay worked relatively well for the fibrinogen-fibronectin interaction when fibrinogen-coated microtiter wells were used for binding and enzyme-labeled antifibronectin was used for detection. I then decided to test whether fibronectin might bind to collagen. I had very little knowledge about collagen at that time other than that it was an abundant connective-tissue protein with a ropelike structure and that it was available for purchase from Sigma. However, when I got the collagen and was ready to do the experiment, which involved coating the microtiter wells with a solution of the collagen, I could not get the protein into solution.

At that time we were running a lot of radioimmunoassays for tumor antigens, and the buffer used for these assays included gelatin (Knox gelatin) to reduce nonspecific interactions and background. It dawned on me that gelatin had something to do with collagen, and I used the radioimmunoassay buffer to coat some wells in my assay plate. The result of the assay was spectacular. Fibronectin appeared to bind exceptionally well to gelatin and much less well, if at all, to native collagen. It was hard to convince my colleagues, though, that this was a specific binding of biological relevance. But after I had completed a purification of fibronectin from plasma using affinity chromatography on gelatin coupled to Sepharose—with unparalleled speed and yield of recovery—there was no doubt about the specificity, and the practical significance of the finding was obvious. Nevertheless, to this day the biological significance of the binding of fibronectin to gelatin rather than to the native collagen is not clear.

Even eating huge quantities of jello does not seem to affect the circulating fibronectin levels. We did that experiment. The purification of fibronectin using the affinity chromatography described in this paper was of great help in fibronectin research,[3] and for this reason the paper has been cited often. On the other hand, because it was so easy to modify the technique by using different agents for elution, many other workers have published their own oft-cited papers and our paper has not been cited as often as the method has been used.

It is exciting to have been participating in the virtual explosion of extracellular matrix research in the last 10 years.[4] It is also rewarding to think that some of the methods we developed helped.

1. Ruoslahti E & Vaheri A. Interaction of soluble fibroblast surface antigen with fibrinogen and fibrin: identity with cold insoluble globulin of human plasma. *J. Exp. Med.* 141:497-501, 1975. (Cited 380 times.)
2. Engvall E & Perlmann P. Enzyme-linked immunosorbent assay, ELISA. III. Quantitation of specific antibodies by enzyme-labeled anti-immunoglobulin in antigen-coated tubes. *J. Immunology* 109:129-35, 1972. (Cited 1,900 times.) [See also: Engvall E. Citation Classic. *Current Contents/Life Sciences* 30(12):16, 23 March 1987.]
3. Ruoslahti E, Engvall E & Hayman E G. Fibronectin: current concepts of its structure and functions. *Collagen Res.* 1:95-128, 1981. (Cited 595 times.) [See also: Ruoslahti E. Citation Classic. *Current Contents/Clinical Medicine* 17(20), 15 May 1989 and *CC/Life Sciences* 32(20), 15 May 1989. In press.]
4. Ruoslahti, E., Pierschbacher, M.D., New Perspectives in Cell Adhesion: RGD and Integrins. Science 238: 491-7, 1987.