This Week's Citation Classic

Deutsch D G & Mertz E T. Plasminogen: purification from human plasma by affinity chromatography. *Science* **170**:1095-6, 1970.
[Department of Biochemistry, Purdue University, Lafayette, IN]

Plasminogen was prepared from human plasma by affinity chromatography on L-lysine coupled to agarose. The single step procedure produced plasminogen in approximately 90 percent yield and the product exhibited multimolecular forms on acrylamide gel electrophoresis. [The *SCI*® indicates that this paper has been cited in over 765 publications since 1970.]

Dale G. Deutsch
Department of Pathology
School of Medicine
and
University Hospital
State University of New York
Stony Brook, NY 11794

August 2, 1983

"This work on plasminogen was performed at Purdue University where I became a graduate student after two years in the Peace Corps. I worked in Edwin Mertz's laboratory where my project was to purify and characterize plasminogen. I succeeded four or five other graduate students who purified plasminogen from any species of animal whose blood they could obtain. The multiple step procedures were very laborious and after about a year of drying out and repacking monstrous columns, I became discouraged and drifted out of the laboratory. My lack of good results made me embarrassed to approach Mertz (who concluded I was goofing off) and he suggested I get a master's degree. To make matters worse, my course work wasn't going well and the chance to help take over the administration building, as part of an antiwar demonstration, came as a welcome relief during this period.

"The idea for the preparation of the affinity adsorbent came about as a result of a series of events. While traveling from Purdue to my home in New York one vacation, I visited my childhood friend, Alan Kaufman, who had started his own medical practice. In our scientific and clinical discussions, he reminded me that ε-aminocaproic acid was used in severe hemorrhage cases (it binds plasminogen and prevents its activation to plasmin[1]) to control bleeding. Some weeks later I thought of hooking up lysine to a column by its α-amino group (which yields an ε-aminocaproic acid functional group) to pull plasminogen out of blood. I mentioned this to my housemate and fellow biochemistry graduate student, George Doellghast. He, in turn, knew of a new procedure which allowed coupling of small ligands by their amino groups to agarose[2] using the CNBr reaction originally described for insolubilizing proteins.[3]

"In the summer of 1969, I worked out the initial method using just 10 ml of human plasma and a small affinity column. When I told Mertz about the new method he immediately recognized its utility and from then on I had his total support and encouragement until I graduated with my PhD. We published an abstract in *Federation Proceedings* and experienced a few setbacks while trying to get it in final form for publication in *Science*. For a while my recovery went from 90 percent to five percent which I discovered was due to switching from phosphate to Tris buffer for washing the column. I also spent weeks trying to measure the purity of the product by a spectrophotometric active site titration method. With the help of John Knox, a fellow graduate student who worked on Mertz's high lysine corn project, we determined that I had phosphate in my enzyme preparation and calcium in my assay buffer and I was measuring the kinetics of calcium phosphate precipitation. A committee at Purdue refused to patent the procedure because they thought it had no commercial value.

"This paper seems to be widely quoted because it is a technique paper which provides a simple and reliable method to prepare plasminogen and to remove plasminogen from other components. Plasminogen plays a central role in the dissolution of blood clots[4] and it is used as a component of other fibrinolytic assays, such as plasminogen activator. Plasminogen itself is studied from a structural point of view and the multiple forms of this proenzyme have now been explained, as described in a recent review article."[5]

1. **Alkjaersig N, Fletcher A P & Sherry S.** ε-Aminocaproic acid: an inhibitor of plasminogen activation. *J. Biol. Chem.* **234**:832-7, 1959.
2. **Cuatrecasas P, Wilchek M & Anfinsen C B.** Selective enzyme purification by affinity chromatography. *Proc. Nat. Acad. Sci. US* **61**:636-43, 1968.
3. **Porath J, Axen R & Ernback S.** Chemical coupling of proteins to agarose. *Nature* **215**:1491-2, 1967. (835 cites.)
4. **Deutsch D G.** An orthomolecular approach to thrombolysis. *Perspect. Biol. Med.* **20**:307-9, 1977.
5. **Castellino F J & Powell J R.** Human plasminogen. *Meth. Enzymology* **80**:365-78, 1981.