DANSYL AND ANTHRANILLOYL HUMAN α -THROMBINS. HIGHLY SENSITIVE ACTIVE SITE PROBES OF THROMBIN CONFORMATION. Y.Y. Shen and L.J. Berliner. Department of Chemistry, The Ohio State University, Columbus, Ohio, U.S.A.

Human α -thrombin was labeled at the active site with the two serine esterase fluorescent probes, dansyl fluoride (Dans) and paranitrophenylanthranilate (Anth). Both derivatives show enhanced quantum yields and blue shifts in their emission maxima. Of most significance is a strong energy coupling to tryptophan (or tyrosine) residues near the catalytic center. Changes in the conformation of the active site region or other changes which alter the environment of the probe will effect the extent of this fluorescence energy transfer between the probe and the protein. Both derivatives, particularly the Anth derivative, show marked changes in their energy coupled spectra upon binding with a small basic ligand such as valerylamine, while Anth shows even greater effects upon association with the prothrombin activation Fragment II. These fluorescent thrombin derivatives are quite powerful in examining thrombin structure alone (α -, β -, and γ -forms) as well as their interactions with other coagulation factors at concentrations more closely approaching serum levels than are attainable with other physical techniques.

EXO-SITE AFFINITY LABELING OF HUMAN α (CLOTTING) AND β/γ (NONCLOTTING) m -[O-2-CHLORO-5-SULFONYLFUOROPHENYLUREIDO)PHENOXYBUTOXY]BENZAMIDINE (mCP(PBA)-F). D.H. Bing, J.W. Fenton, II and M. Cory. Center for Blood Research, Boston, MA; Div. Laboratories and Research, New York State Department of Health, Albany, NY; and SRI, Menlo Park, CA, U.S.A.

Human α -thrombin was selectively and irreversibly inactivated with mCP(PBA)-F (pseudo first order rate constant $k_1 = 3.7 \times 10^{-3} \text{ sec}^{-1}$, 23°). The initial velocities of inactivation followed simple enzyme-substrate kinetics ($K_m(\text{app}) = 72.7 \mu\text{M}$, $k_2 = 3 \times 10^{-3} \text{ sec}^{-1}$). mCP(PBA)-F was determined to be an affinity labeling reagent for both α and β/γ -thrombins (7% α , 20% β and 78% γ) as (1) $[^3\text{H}]m\text{CP(PBA)-F}$ was stoichiometrically incorporated (1.07 mole/mole for α thrombin and 1.19 mole/mole for β/γ -thrombin), (2) $i\text{Pr}_2\text{P-F}$ inactivated α and β/γ -thrombin did not react with mCP(PBA)-F, and (3) the analog o-(2-chloro-5-sulfonylfluorophenylureido)phenoxy benzene lacking the benzamidine, only caused 14% inactivation of α -thrombin. $[^{14}\text{C}]i\text{Pr}_2\text{P-F}$ was only found on the B chain (M_r 32,000) but $[^3\text{H}]m\text{(PBA)-F}$ label was distributed 20% on the A chain and 80% on the B chain in reduced and alkylated α -thrombin electrophoresed in 10% PAGE-SDS-6 M urea. The labeling pattern of β/γ -thrombin with $[^{14}\text{C}]i\text{Pr}_2\text{P-F}$ and $[^3\text{H}]m\text{CP(PBA)-F}$ revealed that ^{14}C label was on the B chain active site fragments of β -thrombin ($M_r \approx 23,000$) and γ -thrombin ($M_r \approx 12,000$), but the ^3H label was on 20% the A chain and 80% on fragments of M_r 6000-10,000. The results suggest that mCP(PBA)-F reacts with residues exclusive of the active site serine within $\sim 10 \text{ \AA}$ radius of the anionic binding site. (Supported by NIH Grants AI 11231, AM 17351, CA 17376, HL 13160 and HL 18825)