0132

EFFECT OF pH ON THE STABILITY AND CONFORMATION OF α-THROMBIN. German B. Villanueva, Department of Biochemistry, New York Medical College, Valhalla, New York 10595.

It is known that storage at pH 6 stabilizes thrombin against inactivation. In order to determine whether structural changes accompany this stabilization, the conformation of human α-thrombin at pH 6.0 and 7.5 was investigated by chemical modification, solvent perturbation, UV difference spectroscopy and circular dichroism. It was shown that the CD spectra of α-thrombin at 230-200 nm peptide region were indistinguishable at two pH values indicating no difference in the secondary structure. However, differences were observed in the 320-250 nm aromatic region suggesting some changes in the microenvironment of the aromatic chromophores. Solvent perturbation in 20% ethylene glycol indicated 3.7 ± 0.5 Trp and 7.8 ± 0.5 Tyr were exposed to the solvent at pH 6.0 while 4.3 ± 0.4 Trp and 8.4 ± 0.5 Tyr were exposed at pH 7.5. Chemical modification of tryptophan residue by dimethyl(2-hydroxy-5-nitrobenzyl)sulfonium bromide in a 100-fold molar excess of the reagent showed 3 reactive residues at pH 6.0 and 6 at pH 7.5. These results suggest that when thrombin is exposed to low pH, structural changes occur that decrease the relative degree of exposure of tryptophan and tyrosine residues. Furthermore, UV difference spectroscopy showed the development of a positive differential spectrum when thrombin at pH 6.0 was exposed to pH 7.5. From this study, it is concluded that the stability of thrombin at pH 6.0 is due to a more compact structure of the enzyme which is probably a result of reduced charge interaction at low pH.

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BIS-ANS BINDING TO HUMAN THROMBINS. G. Metz and L. J. Berliner, Department of Chemistry, The Ohio State University, Columbus, Ohio, U.S.A.

Bis-anilinonaphthalenesulfonate(bis-ANS) binds extremely strongly to the human thrombins accompanied by a much enhanced fluorescence for the dye. One unique feature of bis-ANS binding is an enhancement of TAME hydrolysis by α-thrombin to approximately 175% times the rate for native α-thrombin at pH 8.1 (0 M NaCl). Non-coagulant γ-thrombin is distinguished from highly coagulant α-thrombin by enhancing bis-ANS fluorescence ∿2 times more than that by the latter form. The very strong binding of this dye K_{diss}<10^{-8} M, pH 6.5, 0.05 M phosphate, 0.75 M NaCl) as well as its apparent binding loci situated near, but not obstructing, the catalytic center displays the powerfully useful properties of this probe, extremely sensitive to thrombin environment while not inhibiting its action. This is to be compared with the active site specific fluorophore inhibitor DAPA which prevents binding of other ligands to the thrombin active site.

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CLOTTING AND CHROMOGENIC SUBSTRATE ASSAYS MEASURE SEPARATE THROMBIN ACTIVITIES. M.P. Milad and H.I. Hassouna. Dept of Medicine, College of Human Medicine, Michigan State University, East Lansing, Michigan, U.S.A.

Thrombin, the final serine protease responsible for the conversion of fibrinogen to fibrin, is known to have the ability to cleave other peptides in addition to those involved in blood coagulation. Many synthetic substrates were devised to be acted on by thrombin reflecting esterolytic, amidolytic and other cleaving properties. Antithrombin III, the major protease inhibitor of plasma, has been shown to bind the proteolytic properties of thrombin irreversibly. The purpose of this study was to determine whether all enzymatic properties are destroyed simultaneously or whether the inhibitor binds the active site leaving esterolytic functions intact.

Purified α-thrombin was examined with fibrinogen, and H-D-Phe-Pip-arg-p-nitroanilide assays to prepare standard working curves relating units thrombin to the rate of fibrin endpoint or to the rate of production of chromophore. Then progressive antithrombin inactivation of seryl residue was examined by increasing concentrations of ATIII incubated with thrombin for five minutes and aliquots simultaneously examined for the ability to clot a standard fibrinogen solution and to cleave the synthetic substrate.

The clotting activity decreased steadily with increasing antithrombin, whereas the esterase activity remained constant. The study provides further evidence to the observations of Chang et al (Biochemistry 18: 113, (1979)) that thrombin contains two functional sites, one highly specific for the fibrinopeptide region of fibrinogen and the second site relatively nonspecific, responsible for thrombin's esterolytic activity. An important implication of this study is that clotting assays and chemical assays that use synthetic peptides reflect separate functional properties of the enzyme thrombin.

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THE PRIMARY STRUCTURE OF CROTALASE, A THROMBIN-LIKE VENOM ENZYME, EXHIBITS STRONG HOMOLOGY WITH KALLIKREIN. H. Pirkle, F.S. Markland, S.S. Bajwa, I. Theodor, R. Baumgartner, and H. Kirakossian. Department of Pathology, University of California, Irvine, Calif. and Department of Biochemistry and Cancer Research Institute, University of Southern California, Los Angeles, Calif. U.S.A.

Edman degradation of crotalase and its tryptic and CNBr fragments yielded an N-terminal amino acid sequence, Val-Ile-Gly-Gly-Asp-Glu-Cys-AsN-Ile-AsN-Glu-His-Arg-Phe-Leu-Val-Ala-Leu-Tyr-Asp-Tyr-Trp-Xxx-GlN-Xxx-Phe-Leu-, and internal sequences, -Leu-Ile-Arg-Leu-AsN-Lys-Pro-Val-Ser-Tyr-Ser-Glu-His-Ile-Ala-Pro-Leu-Ser-Leu-Pro-Ser-Ser-Pro-Pro-Ile-Val-Gly-Ser-Val-Cys-Arg-Ala-Met-Gly-Trp-Gly-GlN-Thr-Thr-Ser-Pro-GlN-Glu-Thr-Leu-Pro-Asp-Val-Pro-His-Cys-Ala-AsN-Ile-AsN-Leu-Leu-Asp-Tyr-Glu-Val-Cys- and -Ser-Val-GlN-Phe-Asp-Lys-Glu-GlN-GlN-Arg-. The longer internal sequence aligns homologously with positions 105-168 of chymotrypsinogen with gaps, each 5 residues long, following Pro 128 and GlN 143. No homologous alignment has been found for the shorter internal sequence. The total percentages of identical residues in homologous sequences of some other serine proteases are:

kallikrein	36%	chymotrypsin	22%	plasmin	22%
trypsin	32%	elastase	26%	factor X	21%
thrombin	29%	bacterial trypsin	22%	factor IX	16%

These findings led to experiments which demonstrated that crotalase has specific enzymatic properties resembling kallikrein.