

SUBSTRATE SPECIFICITY OF BOVINE THROMBIN AND FACTOR Xa TOWARD PEPTIDE PARA-NITROANILIDE SUBSTRATES. Richard Lottenberg and Craig M. Jackson, Department of Biological Chemistry, Washington University School of Medicine, St. Louis, Missouri, U.S.A.

Kinetic parameters were obtained for commercially available chromogenic substrates with bovine alpha thrombin and Factor Xa. Enzyme concentrations were determined by active site titration using p-nitrophenyl-p-guanidinobenzoate. Initial rate studies were performed at 25 deg. C at pH 7.8. Non-linear least squares procedures were utilized to determine Michaelis constants (K_m 's) and catalytic constants (k_{cat} 's) for an assumed simple Michaelis-Menten model for the hydrolytic reaction. For several substrates with very high K_m 's, the second order rate constant, k_{cat}/K_m , was the only parameter that could be accurately obtained, although estimates for the two kinetic parameters could be calculated from linearized forms of the Michaelis-Menten equation. Twenty peptide-anilide substrates were evaluated with thrombin. For those substrates for which completely valid parameters could be determined, K_m estimates ranged from 0.43 to 360 μM ; k_{cat} values varied from 0.060 to 130/second. The k_{cat}/K_m values ranged from 500 to 93,000,000 liter/mole/second. Twenty-one peptide-anilide substrates were evaluated with Factor Xa. The K_m range was 35 to 1300 μM ; k_{cat} range 0.071 to 260/second. The k_{cat}/K_m values ranged from 15 to 1,900,000 liter/mole/second. Effects of pH, ionic strength, temperature and concentration of polyethylene glycol in the reaction mixture were also determined.

Inferences regarding the substrate specificity of thrombin and Factor Xa that can be drawn from these data will be discussed.

THE PROTHROMBIN TIME: THE MECHANISM OF IN VITRO ACTIVATION. R. Palmer and H. Gralnick. National Institutes of Health, Bethesda, Maryland USA.

We have described shortening of the prothrombin time (PT) when blood is collected in borosilicate or siliconized borosilicate tubes. The shortening is time dependent and occurs more rapidly at 4°C than at room temperature. We have studied the mechanisms of in vitro activation of the PT utilizing normal blood and blood congenitally deficient in coagulation factors. We have found that the blood deficient in factor XI has the same rate of shortening of the PT as in normal blood, while blood deficient in factor IX has only 50% that of normal and factor XII deficient blood does not have any in vitro activation. The blood deficient in C1 INH has the most severe shortening of the PT.

Analysis of the coagulation factors affected during the in vitro activation of normal blood revealed that factor VII-X is increased and this is related primarily to factor VII activation. In borosilicate factor V did not change, factor IX decreased by 15-20% while factor XII showed no change in the assay system. The activation can be totally blocked by addition of C1 INH or corn trypsin inhibitor (specific inhibitor of XII activation) to whole blood. These studies indicate that the PT is shortened in vitro by at least two mechanisms of activation of whole blood: 1) factor XII is activated by surface contact with siliconized borosilicate or borosilicate and in turn, directly activates factor VII; 2) factor XIIa activates prekallikrein to kallikrein which in turn activates factor IX. Factor IXa then activates factor VII and it would appear that approximately one-half of the activation of the PT is directly through this pathway. Inhibitors of factor XII activation, C1 INH and CFI can totally inhibit the activation in vitro. Thus, surface activation of factor XII is the pivotal reaction in the in vitro shortening of the PT in whole blood. These studies allow new strategies for the prevention of in vitro activation of the PT, and may allow for more precise measurement of the PT without concern for contact activation by the use of specific inhibitors.

KINETIC DESCRIPTION OF CONVERSION OF BOVINE PRETHROMBIN 2 TO THROMBIN. Thomas L. Carlisle and Craig M. Jackson, Department of Biological Chemistry, Washington University School of Medicine, St. Louis, Missouri, U.S.A.

Bovine Factor Xa slowly converts Prethrombin 1 to thrombin plus Fragment 2. Inclusion of Ca^{2+} increased the rates of Prethrombin 1 consumption, Prethrombin 2 production and thrombin formation detected by SDS polyacrylamide gel electrophoresis or by thrombin assay. Ca^{2+} also increased the rate of thrombin formation from equimolar mixtures of Prethrombin 2 and Fragment 2 (Prethrombin 2/Fragment 2) by approximately 1.8 fold. Calcium ion thus increases the rate of both proteolyses required to generate thrombin from Prethrombin 1. Studies using Factor Xa (des light chain residues 1-44) indicated that this effect of Ca^{2+} required the region of Factor Xa containing gamma-carboxyglutamic acid.

Factor Va markedly lowered the apparent K_m of Factor Xa for Prethrombin 2/Fragment 2, with decreases greater than 20 fold observed under some conditions. The apparent maximum velocity also increased by up to 50 fold. The extent of increase was greater at higher concentrations of Factor Va, and was about 6 fold greater in the presence of Ca^{2+} than in its absence. Factor Va binding to Factor Xa (forming XaVa with enhanced substrate binding and/or catalytic efficiency), and Factor Va binding to Prethrombin 2/Fragment 2 (forming a substrate more readily bound and/or cleaved) must be considered among the possible explanations for these effects. Previous qualitative observations suggest that these effects of Factor Va on activation of Prethrombin 2/Fragment 2 are important in understanding the activation of prothrombin.

INFLUENCE OF COD LIVER OIL AND CORN OIL ON PLATELETS AND VESSEL WALL IN MAN. A. Nordøy, J.H. Brox and J.E. Killie; Department of Medicine, University of Tromsø, 9012 Tromsø, Norway

Two groups of 10 healthy males were given dietary daily supplement of 25 ml cod liver oil (CLO) or corn oil (CO) for periods of 6 weeks in a blind crossover study. Between the two periods and after the last period there were 3 weeks and 4 months, respectively, without supplement. Plasma lipids, platelet lipids, prostaglandins and platelet functions were examined. Pieces of superficial forearm veins were excised after the two dietary periods and assayed for prostacyclins by bioassay and by GLC-MS.

Collagen-induced platelet aggregation and TXB₂ formation were significantly reduced in both the CLO and CO group. CLO was more potent than CO. The MDA production was not affected.

In the total fatty acids of plasma and in the main platelet phospholipid fractions, CLO increased the amounts of fatty acids of the ω -3 family whereas the ω -6 fatty acids decreased. CO only moderately influenced the composition of these lipids. Cholesterol and triglycerides levels in serum were not affected. The vein biopsies showed no significant production of prostacyclin-like material in any of the dietary groups. In summary small amounts of CLO added to the diet gives significant changes in platelet lipids and reduced platelet aggregation and TXB₂ production.