Prethrombin-1, the gla-domainless prothrombin intermediate, is activated efficiently to thrombin by Prothrombinase assembled on the activated platelet surface.

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Prothrombinase, a Ca^{2+}-dependent, 1:1 complex of factors Va and Xa bound to activated platelets, effects prothrombin cleavage at Arg271 to form prethrombin-2 followed by cleavage at Arg320 to yield thrombin. In contrast, initial cleavage at Arg320, to form the meizothrombin intermediate, followed by cleavage at Arg271, is the preferred pathway for thrombin formation via Prothrombinase assembled on PCPS vesicles (25%PS, 75%PC). Thus, procoagulant activity is optimized physiologically as formation of meizothrombin, a protease with substantial anticoagulant activity, is avoided on the activated platelet surface.

Previous studies defining the order of bond cleavage catalyzed by Prothrombinase have been done in the presence of thrombin inhibitors to prevent thrombin-catalyzed cleavage of prothrombin or its intermediates at Arg155 or Arg 284. To determine if thrombin-derived intermediates are formed and effectively processed, cleavage of prothrombin (1.4 μM) was monitored, in the absence of thrombin inhibitors, in two conditions: 1) following the simultaneous addition of 0.1mg/mL collagen and 5nM factor Xa to human platelets ($3x10^8$/mL), or 2) in an assay system containing 0.5nM factor Va, 5nM factor Xa and 20μM PCPS vesicles. As the amount of prethrombin-1 formed and subsequently cleaved was substantially different in both systems, analyses were done to define both the kinetic constants governing its cleavage to form thrombin, and the order of bond cleavage utilized, on both activated platelets and PCPS vesicles.

The prethrombin-1 generated in the vesicle system was a poorer substrate for Prothrombinase, accumulated significantly, and persisted, consistent with the reported, and substantiated, 6 μM Km. In marked contrast, the prethrombin-1 generated on the activated platelet surface approached only 0.2 μM, but was rapidly cleaved at Arg271 and Arg320 to yield thrombin. Determination of the kinetic constants for prethrombin-1 activation by Prothrombinase bound to activated platelets from two donors yielded an apparent Km = 3.6 μM or 3.9 μM, and a Vmax = 1.3 nM/sec or 3.6 nM/sec, respectively. These observed Km values are only ~1.5-fold lower than those observed in a vesicle system, and are inconsistent with the rapid turnover of prethrombin-1 that is generated *in situ* by thrombin formed at the activated platelet surface.

We hypothesize that during prothrombin activation *in vivo*, the thrombin formed initially, and any prethrombin-1 formed subsequently, are retained at or near the platelet surface, thus increasing their local concentrations and allowing for the effective conversion of prethrombin-1 to thrombin. The ability of activated platelets to avoid meizothrombin formation during prothrombin activation and to effect the rapid conversion of any prethrombin-1, formed subsequently by thrombin-catalyzed cleavage of prothrombin, are activities that optimize their procoagulant function.