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

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ABSTRACT

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. Procoagulant activity is optimized as generation of the meizothrombin intermediate, a protease with anticoagulant activity, is avoided. In these studies using 1.4 μM prothrombin, the thrombin formed by Prothrombinase, assembled on either activated platelets or PCPS vesicles, cleaved prothrombin at Arg155 to yield the Gla-domainless prethrombin-1 intermediate. The prethrombin-1 generated in the vesicle system was a poorer substrate for Prothrombinase and accumulated significantly, consistent with the reported 6 μM K_m. In marked contrast, the prethrombin-1 generated on the activated platelet surface approached 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 K_m equal to 3.6 μM and 3.9 μM and a V_{max} equal to 1.3 nM/sec and 3.6 nM/sec, respectively. These observed K_m values are only about 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