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Kevin R. Siebenlist
Marquette University, kevin.siebenlist@marquette.edu

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Fibrinogen Containing γ' Chains; Is the Assay Measuring What Farrell's Group Expects?

Kevin R. Siebenlist
Department of Biomedical Sciences, College of Health Sciences, Marquette University, Milwaukee, WI

Dr Farrell has objected to certain findings in our paper[1] that do not agree with his published work[2]: for example, in our hands, factor XIII (fXIII) activation in the presence of fibrinogen 2 (γ_A-γ') was slower than with fibrinogen 1 (γ_A-γ_A). To explain the differences, we suggested that the fibrinogen 2 used in his experiments was likely to have contained fXIII that had coeluted with the fibrinogen 2.As far as we can determine, Farrell's group did not process the fibrinogen 2 in a manner that would have removed the fXIII present in chromatographically isolated fibrinogen 2.[3] Contrary to Dr Farrell's assertion, we very carefully redescribed our method for rendering fibrinogen 2 fXIII free and how the product was analyzed and shown to be fXIII free.[1,3]

Farrell asserted that his "activity" assay for fXIII activation was more appropriate than activation peptide cleavage from fXIII. Activation peptide release is an accepted and often-used surrogate for measuring fXIII activation. Furthermore, activation peptide release is the rate limiting step of fXIII activation, not the Ca^{++}-mediated dissociation of A* from the B subunits.

To support the contention that the fibrinogen 2 Farrell's group used was depleted of fXIII, he included some unpublished experiments in his letter. The methodological design of these experiments raises more questions than they answer. First, since chromatographic separation of fibrinogen 1 from fibrinogen 2 results in fXIII-free fibrinogen 1, why was transglutaminase activity observed with fibrinogen 1 alone? Second, we previously demonstrated that “unactivated” fXIII, such as is present in chromatographically purified fibrinogen 2 and in the activation mixtures that Farrell measured in his assay, cross-links fibrin almost as rapidly as thrombin-activated factor XIIIa. Factor XIII also cross-links fibrinogen and artificial substrates, but at slower rates.[4] Since only one (ie, biotinylated pentylamine incorporation into dimethylcasein) of several reactions (eg, fibrin(ogen) cross-linking, dimethylamine incorporation into fibrin(ogen), biotinylated pentylamine incorporation into fibrin(ogen)) was measured in his assay, we wonder what the effect of competing fXIII substrate(s), namely fibrin(ogen), was on the pentylamine incorporation into dimethylcasein?

Dr Farrell also posited that the discrepancies in FPA release between our paper and one by Cooper et al[5] might be explained by contaminants or variable degrees of fibrinogen degradation in our preparations. Our explanation is simpler. Differences between our results and those of Cooper et al[5] were due to the lower levels of thrombin we employed, and those lower concentrations enabled us to measure differences in FPA release between fibrinogen 2 and fibrinogen 1. Other experiments in our paper, showing down-regulation of thrombin catalytic activity (k_{cat}) when bound to fibrin 2 γ′ chains, provide more than adequate support for our explanation and also for the slower release of activation peptide from fXIII in the presence of fibrinogen 2.

References

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