This paper describes the localization of four secreted platelet proteins (platelet factor 4, β-thromboglobulin, fibrinogen, and the platelet-derived growth factor) to the α-granule fraction of platelets. The ability of several agents to induce secretion of α-granule proteins and the effects of cyclooxygenase inhibitors on secretion are also described. [The SCOP indicates that this paper has been cited in over 215 publications.]

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My interest in secreted platelet proteins began when I joined the laboratory of the late Hymie Nossel. My task in the laboratory was to develop a radioimmunoassay for platelet factor 4 (PF4),2 to use together with the radioimmunoassay for fibrinogen to try to detect thrombotic events in patients. We felt that a measure of platelet activation might add sensitivity and specificity.

When we first isolated the secreted platelet protein, it was described, β-thromboglobulin (β-TG),2 this protein was purified in our laboratory as well, and specific antibodies against each of them were developed for both PF4 and β-TG.3 Platelet α-granules have recently been distinguished from platelet lysosomes,4 and there was a report that PF4 and fibrinogen were localized in the α-granules.5 In that report, however, PF4 was assayed by its heparin-neutralizing activity, and it was not clear whether other platelet proteins might contribute to the heparin-neutralizing activity.

Thus, with specific assays for PF4 and β-TG available, it was decided to investigate their subcellular localization, using the method of subcellular fractionation developed by Broekman et al.6 Han Broekman agreed to collaborate in such a study, so we proceeded to isolate and fractionate platelets and to assay the various fractions for PF4 and β-TG. Additionally, we were able to thrombin-treat the fractions and to assay them for fibrinopeptide A as a marker of fibrinogen. Arthur Chernoff, working in the laboratory of DeWitt Goodman at Columbia, assayed the fractions for platelet-derived growth factor activity. The large proteins were shown to be localized in the fractions that were rich in α-granules, and those fractions were distinct from the dense granule fractions that contained serotonin and from the lysosomes that contained acid hydrolases.

We were also interested in determining the relationship between secretion from the dense granules and the α-granules and in comparing the characteristics of release from these granules with published reports of secretion from lysosomes. Our studies showed that α-granule release induced by thrombin, collagen, and the cyclic endoperoxide analogue (U46619) occurred at lower concentrations of agonists than did dense granule release. Since published studies had demonstrated that lysosomal enzyme release required higher concentrations of agonists than did dense granule release, it appeared that α-granules were most sensitive to release, with dense granules next, and lysosomes least sensitive.

Finally, the effect of cyclooxygenase inhibitors on α-granule release was examined. Indomethacin added to gel-filtered platelets inhibited secretion of α-granule proteins induced by low concentrations of thrombin or collagen but not by high concentrations of these agonists. Similarly, secretion induced by collagen or thrombin was only partially inhibited in platelet-rich plasma prepared from subjects who had ingested aspirin, indicating complete dependence of α-granule secretion induced by these agents on cyclooxygenase activity. Aspirin ingested by normal volunteers caused a small but significant decrease in the mean plasma β-TG level but no significant change in the mean PF4 level, suggesting that in vivo release is likely to be induced by agents such as thrombin and collagen.

It is likely that this paper has been cited frequently because it was the first to demonstrate definitively the localization of these important secreted platelet proteins in the α-granules and to begin to characterize the differences between dense granule and α-granule secretion. Subsequently, several papers have confirmed these findings, and recently there have been a number of excellent papers that have used immunoelectron microscopy to visualize individual proteins within α-granules.