

REVIEW ARTICLE

Plasma Levels of β -Thromboglobulin and Platelet Factor 4 as Indices of Platelet Activation In Vivo

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Measurement of plasma levels of two secreted platelet proteins (β -thromboglobulin and platelet factor 4) has been suggested as a means for detecting increased platelet activation in vivo. A crucial question in the measurement is the distinction between in vivo and in vitro secretion of the proteins. One approach to this distinction is the measurement of both proteins in each sample. These proteins are present in platelets in similar amounts and are released in similar quantities, but the plasma levels of β -thromboglobulin exceed the plasma levels of platelet factor 4. This difference in plasma level is presumably due to more rapid removal of platelet factor 4 from the plasma

level, and there is suggestive evidence that the rapid removal of released platelet factor 4 is due to its binding to endothelial cells. It appears that when there is increased release of β -thromboglobulin and platelet factor 4 in vivo, there is an increase in the ratio of plasma β -thromboglobulin to plasma platelet factor 4 compared to that found in normal individuals, whereas when in vitro release is responsible for elevated levels, the ratio decreases. Thus measurements of both proteins in each blood sample will allow distinction between in vivo release and artefactual in vitro release.

PLATELETS play an important role in normal hemostasis by forming aggregates that seal defects in the vessel wall. Pathologic extension of platelet aggregates may, however, contribute to the development of occlusive vascular disease. Drugs that inhibit platelet aggregation should prevent extension and inhibit the development of occlusive vascular disease. In this regard, recent studies have claimed effectiveness for antiplatelet agents in improving the outcome in patients with cerebral^{1,2} or coronary arterial disease.^{3,4} It seems likely that antiplatelet agents would be most effective in patients with increased platelet reactivity and thus tests that could identify increased reactivity might guide therapy in individual patients. Aggregation tests have been particularly useful in studying bleeding disorders due to defects in platelet function and in the monitoring of pharmacologic agents, but have not been particularly useful in identifying abnormalities in thrombotic disorders. Shortened platelet survival has been reported in some patients with coronary artery disease^{5,6} and homocystinemia,⁷ but the differences from normal are small and not always reproducible.⁸ Since these methods have significant limitations, it has been suggested that measurements of released platelet materials might provide a better index of platelet activation in atherosclerotic vascular disease and arterial thrombosis.

Methods have recently been developed for radioimmunoassay of β -thromboglobulin and platelet factor 4, two platelet-specific proteins that are secreted from the α -granules during the release reaction induced by ADP, epinephrine, arachidonic acid, collagen, and thrombin.⁹⁻¹⁵ The reagents needed for radioimmunoassay of these proteins are now commercially available. Additionally, Rucinski and colleagues have reported a radioimmunoassay for low-affinity platelet factor 4, a protein that appears to be the precursor of β -thromboglobulin in platelets.^{16,17} Despite the name low-affinity platelet factor 4, this protein is structurally and immunologically distinct from platelet factor 4.¹⁸ With the potential importance of these assays for secreted platelet proteins in identifying patients with increased

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platelet release *in vivo*, there are likely to be many studies involving measurement of one or more of these proteins in various clinical situations with the results of measurements used to initiate and follow therapy. Thus it seems appropriate to review the use of these assays in clinical studies, with special attention to the validity of such measurements as indices of *in vivo* platelet release.

β -thromboglobulin, the first platelet-specific protein for which a radioimmunoassay was reported, is secreted from platelets as a tetramer of 4 identical subunits of molecular weight 8850,¹⁸ thus the tetrameric molecular weight is 35,400. β -thromboglobulin appears to be a proteolytic product of low-affinity platelet factor 4, resulting from cleavage of a tetrapeptide from the N-terminal end of each subunit of low-affinity platelet factor 4.¹⁷ At the present time, β -thromboglobulin and low-affinity platelet factor 4 cannot be distinguished immunologically.¹⁶ Platelet factor 4 is also tetrameric. However, it is secreted as a complex of two tetramers of PF4 and two molecules of proteoglycan carrier with a combined molecular weight of 350,000.^{19,20} The complex apparently dissociates in plasma. The molecular weight of the platelet factor 4 tetramer is 30,800^{19,20} and it, like β -thromboglobulin, is composed of four identical subunits, in this case of molecular weight 7700.²¹⁻²⁴ The reported radioimmunoassays have been developed for platelet factor 4 rather than the platelet factor 4-carrier complex.

The platelet content of these two proteins is quite similar. The mean content of platelet factor 4 in a previously reported study was 18.0 ± 2.0 (SEM) $\mu\text{g}/10^9$ platelets ($n = 14$), and the mean content of β -thromboglobulin was 17.7 ± 1.6 (SEM) $\mu\text{g}/10^9$ platelets ($n = 14$).²⁵ In any individual the platelet content of either protein may exceed the content of the other protein. Studies of *in vitro* release of β -thromboglobulin and platelet factor 4 have shown that release of β -thromboglobulin is equal in quantity and rate to release of platelet factor 4 whether studies are done in citrated platelet rich plasma,^{9,10} with gel-filtered platelets in Tyrode's buffer,^{9,10} or with whole blood without anticoagulant.^{9,26,27}

Dawes and colleagues reported on the clearance of platelet factor 4 and β -thromboglobulin when 200 ml of serum containing these proteins was infused into a normal volunteer. In this situation the clearance of PF4 was extremely rapid and the $t_{1/2}$ could not be accurately determined, while the clearance of β -thromboglobulin had a $t_{1/2}$ of 100 min.²⁸ Caution must be used in interpreting the results of a serum infusion because of possible artefacts, e.g., release of endogenous platelet factor 4 or β -thromboglobulin by active

thrombin in the serum. More recently Musial and colleagues²⁹ have studied the clearance of purified human PF4 and low-affinity PF4 in rhesus monkeys and have reported that biphasic exponential curves are found for the decay of both proteins, with half-disappearance times for the fast and slow components for platelet factor 4 of 2.1 and 70 min and for low affinity platelet factor 4 of 8.4 and 63 min. Clearance of purified materials in humans has not been studied. It is possible that the more rapid initial clearance of platelet factor 4 in the studies reported by Dawes and by Musial is due to its binding to endothelial cells. Such binding has been demonstrated directly *in vitro* with human platelet factor 4 and cultured human endothelial cells³⁰ and inferred *in vivo* from the immediate increase in the plasma level of platelet factor 4 that follows infusion of heparin into normal individuals.²⁸ We have observed that heparin added to whole blood *in vitro* does not increase the plasma level of platelet factor 4, suggesting that the increase in platelet factor 4 in the infusion study is due to release of platelet factor 4 bound to vascular endothelial cells rather than release from blood cells (unpublished observations). β -thromboglobulin has been reported to bind to bovine endothelial cells *in vitro*,³¹ but the reported affinity is such that in humans only small amounts would be bound *in vivo* in relationship to the plasma concentration under physiologic circumstances, if the binding affinity for human endothelial cells is similar to that for bovine cells.

Guzzo et al. have recently presented evidence that low affinity platelet factor 4/ β -thromboglobulin is metabolized by the kidney, and that elevated levels of this protein are found in the plasma of patients with chronic renal failure. Platelet factor 4 levels were normal in their patients with chronic renal failure.³² A correlation between plasma β -thromboglobulin and serum creatinine was also noted by Ludlam and Anderton³³ and by Depperman and colleagues.³⁴

Thus, platelet factor 4 and β -thromboglobulin are present in similar quantities within platelets and they are released in similar quantities *in vitro*, however, the two proteins are cleared from plasma at different rates, possibly because of rapid binding of released platelet factor 4 to endothelial cells. What, then, is the expected relationship of β -thromboglobulin and platelet factor 4 levels in plasma from normal individuals and from patients with increased *in vivo* release? The distributions of levels of the two proteins in normals from our laboratory have recently been published.^{35,36} Both distributions were logarithmic. The median value for β TG was 17.8 ng/ml with 90% of the values between 6.6 ng/ml and 47.9 ng/ml. The median value for PF4 was 6.0 ng/ml with 90% of the values between

1.7 ng/ml and 20.9 ng/ml. Musial and colleagues have suggested that the ratio of low-affinity platelet factor 4 to platelet factor 4 helps in distinguishing in vivo release from in vitro, with higher ratios favoring in vivo release.²⁹ The ratios of β -thromboglobulin to platelet factor 4 calculated from our normal values are 3.9 for the lowest levels in the distribution, 3.0 for the median values, and 2.3 for the highest levels. These ratios would suggest that there is a progressively greater contribution of in vitro release as the measured levels in normal individuals increase, and that better methods of preventing in vitro release need to be developed. How differences in platelet counts might affect the β -thromboglobulin to platelet factor 4 ratio is not known.

Can the concept of the β -thromboglobulin to platelet factor 4 ratio be used in evaluating clinical studies for in vitro versus in vivo release? When blood from a single venepuncture was collected into a series of vacutainer tubes, the plasma concentrations of both β -thromboglobulin and platelet factor 4 increased in successive samples.^{35,36} The initial levels showed a β -thromboglobulin to platelet factor 4 ratio of 3.0, while in the 14th tube the ratio was only 1.3, suggesting a progressive contribution of in vitro release in this system. In a recent study of patients undergoing hypertonic saline induced abortion, Nossel and colleagues³⁷ reported that plasma β -thromboglobulin levels changed dramatically following hypertonic saline infusion into the uterus, while changes in plasma platelet factor 4 levels were much smaller. The mean β -thromboglobulin to mean platelet factor 4 ratio in the pre-infusion samples was 2.3 and following hypertonic saline infusion the ratio increased to 3.7 at 1 hr after saline infusion, 5.0 at 2 hr, and 5.9 at 4 hr. The β -thromboglobulin to platelet factor 4 ratio then fell so that at 24 and 48 hr after saline infusion it was 1.8 and 2.0, similar to the initial ratio. These values suggest that the increases in β -thromboglobulin and platelet factor 4 levels in the early postinfusion samples largely reflect in vivo release.

Thus far the only circumstance in which platelet factor 4 levels have markedly exceeded β -throm-

Table 1. Plasma Levels of β TG and PF4 in Clinical Situations

	β TG	PF4
Normal individuals	Normal	Normal
In vivo release	Increased	Normal or slightly increased
In vitro release	Increased	Increased
In vivo heparin	Normal	Markedly increased
Renal failure	Increased	Normal

boglobulin levels in our laboratory is following the injection of heparin. This has been noted also by Dawes and colleagues.²⁸ In our experience, heparin infusion gives rise to platelet factor 4 levels of 300–400 ng/ml with no change in the level of β -thromboglobulin (unpublished observations). This increase in platelet factor 4 concentration in plasma following heparin infusion has been attributed to mobilization of platelet factor 4 from binding sites on the vascular endothelium.³⁰

We conclude that a normal level of β -thromboglobulin is good evidence that increased platelet activation is not occurring in vivo. A high plasma level of β -thromboglobulin can be equated with increased in vivo platelet release only if in vitro release is ruled out and if the serum creatinine is normal. One method to rule out in vitro release is to measure the plasma level of platelet factor 4 in the same sample. Normal or only slightly elevated levels of platelet factor 4, with high β -thromboglobulin levels strongly suggest in vivo release, while comparable elevations in both proteins in the absence of heparin therapy suggest in vitro release. Thus, studies in which only one of the proteins has been measured and in which elevations are found in the plasma levels of that protein, are difficult to interpret because there is no control for in vitro release. These relationships are summarized in Table 1.

Finally, while plasma levels of β -thromboglobulin and platelet factor 4 can be measured and the values can be used in assessing in vivo platelet release, it must be remembered that the functions of these proteins are not known, either in the platelet α -granules or in the plasma following release.

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