HYPOTHESIS

Studies on Human Platelets III. A Contractile Protein Model for Platelet Aggregation

By F. M. BOOYSE AND MAX E. RAFFEISON, JR.

S INCE THE DISCOVERY of the actomyosin-like contractile protein, thrombosthenin, a vast new area of research has been introduced in the study of platelets.\(^1\)\(^-\)\(^2\) It has been suggested that this protein may, in some manner, be involved in platelet aggregation and/or clot retraction.\(^3\)\(^-\)\(^6\) An evaluation and comparison of the properties of contractile proteins with the reactions and factors involved in platelet aggregation suggested to us that platelet aggregation may be mediated by a surface or near surface localized actomyosin-like protein. The purpose of this communication is to propose a hypothetical molecular model for platelet aggregation, directly involving the participation of a surface or near surface localized actomyosin-like protein.

An actomyosin-like protein (thrombosthenin) with typical contractile protein properties has been isolated from platelets.\(^1\)\(^-\)\(^2\) This protein exhibits ATPase activity, consists of myosin-like and actin-like moieties,\(^1\)\(^7\) and constitutes 15–20 per cent of the total platelet protein.\(^1\)\(^-\)\(^2\) In addition, several investigators have presented data which may be interpreted as suggesting the surface or near surface location of at least part of this actomyosin-like protein. Nachman et al. have isolated thrombosthenin from purified platelet membranes;\(^8\) kinetic studies of Chambers et al. have indicated that the properties of the ecto-ATPase of intact platelets and those of isolated thrombosthenin are very similar, if not identical;\(^9\) thrombosthenin antiserum inhibited platelet ecto-ATPase activity and also induced platelet agglutination,\(^10\) suggesting the formation of a surface localized antigen-antibody complex, and finally the surface location indicated by the thrombosthenin-fluorescent antibody technic.\(^8\)
CONTRACTILE PROTEIN MODEL

Hypothesis for Platelet Aggregation

The following mechanism, based upon the known properties of contractile proteins, would seem to accommodate and provide a basis for explanation of the reactions involved in platelet aggregation:

(a) A surface or near surface localized (exposed to environment) platelet actomyosin-like contractile protein (thrombosthenin?) catalyzes the splitting of ATP.

\[ \text{ATP} \rightarrow \text{ADP} + \text{Pi} \]

The concentration of available substrate (ATP) is, however, below the critical concentration required to dissociate the platelet actomyosin complex into its component parts, platelet F-actin and platelet myosin.\(^1\)

(b) Under the influence of a physiologic stimulus such as thrombin, which stimulates glycolysis,\(^11\) the concentration and availability of ATP is greatly increased. The ATP concentration is increased to that required for dissociation, resulting in the dissociation of the platelet actomyosin complex into platelet F-actin and linear aggregates of platelet myosin.\(^12\)

(c) In the dissociated form the ATPase activity is low, i.e., that of platelet myosin alone.\(^1\) As the ATP concentration is decreased due to the ATPase activity of the platelet myosin, reassociation of the dissociated moieties begins and the ATPase activity rises as the MgATPase (or CaATPase) of the platelet actomyosin complex evidences itself.

(d) This reassociation, however, does not merely involve intramolecular interactions between protein moieties on a single platelet, but extensive intermolecular interaction between platelet F-actin and platelet myosin on adjacent platelets.

(e) These intermolecular platelet actomyosin bridges are then able to contract in the presence of ATP (at concentrations below that required for dissociation of complex), drawing the platelets into a tight mass or thrombus.

A schematic illustration of the model is given in Figure 1.

DISCUSSION

According to the proposed model, the driving force in platelet aggregation is the reversible dissociation of a platelet actomyosin-like complex exposed to the environment. Conditions that affect the interaction and dissociation of contractile proteins have been well documented.\(^13\) A definite set of interrelated, well-defined conditions are required to maintain the integrity of the actomyosin complex. It is therefore conceivable that any reactions and/or factors interfering with the steady state conditions required to maintain the integrity of this complex will result in alterations in the interaction process and subsequently be reflected by alterations in platelet adhesiveness.

For example:

(1) The concentration of free calcium has a key role in the actin-myosin interaction process. Several reports have indicated that MgATP is the moiety that controls the dissociation of actin and myosin by altering the level of calcium needed for association.\(^14\)-\(^15\) Removal of either calcium and/or magnesium with chelating agents such as EDTA, citrate and oxalate would result in
dissociation of the actomyosin complex, thereby preventing the formation of intermolecular platelet actomyosin bridges and aggregation.

2) Sulfhydryl blocking reagents have the ability not only to block ATPase activity, but also -SH groups required for actin-myosin interactions. These blocking agents would be expected to prevent or inhibit aggregation due to the inability to form intermolecular protein bridges.

3) Addition of ADP would result in competitive inhibition of the enzyme reaction (ATPase activity) causing an accumulation of ATP substrate and subsequent initiation of both the dissociation and aggregation processes.

4) Finally, the addition of ATP to actomyosin solutions causes a marked decrease in both viscosity and intensity of light scattered by the actomyosin solutions. These decreases have been associated with the dissociation of the actomyosin complex into its component parts. ATP acts here as a competitor with actin for combination with myosin. Born et al. have shown that a large number of adenosine analogues are active as inhibitors of aggregation. Analogues belonging to this group, as well as analogues described by Ikehara et al., however, have been shown to decrease drastically the viscosity and intensity of light scattered by the treated actomyosin solutions. This, presumably is due to dissociation of the actomyosin complex by the specific analogues. Related compounds such as AMP and adenosine could have a similar dissociating ability, thereby inhibiting platelet aggregation.

The proposed model provides a molecular basis for the attachment of one platelet to another, is consistent with the general reactions and factors involved in platelet aggregation, and appears to provide a reasonable explanation for the data available at this time.
CONTRACTILE PROTEIN MODEL

SUMMARY

A new hypothesis is proposed for platelet aggregation. The model involves the formation of *intermolecular* platelet actomyosin bridges between platelet actin and platelet myosin moieties localized on or near the surface of adjacent platelets. These *intermolecular* platelet actomyosin bridges can then contract in the presence of ATP, drawing the platelets into a tight mass or thrombus.

SUMMARIO IN INTERLINGUA

Es proponite un nove hypothese pro explicar le mechanismo del aggregation de plachettas. Le modello include pontes intermolecular de actomyosina plachettal formate inter myosina plachettal e actina plachettal localisate al o proxime al superficie de plachettas adjacente. Iste pontes intermolecular de actomyosina plachettal se contrahe in le presentia de ATP con le resultato del fusion de plachettas ad in un massa coherente, i.e., un thrombo.

REFERENCES

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