Platelet and Plasma Fibrinogens Are Identical Gene Products

Abstract. *Human platelet fibrinogen has been shown to be indistinguishable from plasma fibrinogen by a variety of criteria, including subunit composition and cross-linking characteristics as judged by sodium dodecyl sulfate–gel electrophoresis, quantitative amino terminal analysis of fibrin, and the amino acid compositions of fibrinopeptides. The structural identity of the fibrinopeptides released from platelet and plasma fibrinogens is consistent with the two proteins being products of the same gene or genes.*

Mammalian blood platelets contain a thrombin-clottable protein (platelet fibrinogen) independent of any plasma fibrinogen adsorbed to the external surface of the platelet (*1–4*). Although platelet fibrinogen cross-reacts with antibodies produced against plasma fibrinogen (*1*, *2*), previous reports have indicated that platelet fibrinogen differs significantly from plasma fibrinogen with regard to its hydrodynamic properties (*2*, *3*), mobility on sodium dodecyl sulfate (SDS)–gels (*2*), clottability by thrombin, and susceptibility to attack by plasmin (*2*, *5*). Some investigators (*6*) have attributed the bulk of these differences—if not all of them—to degradation of the fibrinogen by the highly active proteolytic systems in platelets, whereas others (*2*, *5*) have indicated that the two fibrinogen molecules are fundamentally different in origin as well as in structure. Accordingly we have examined the amino acid compositions of the fibrinopeptides A and B from platelet fibrinogen to see if amino acid sequence differences exist between the two types. Since fibrinopeptides are highly variable from species to species (*7*), we anticipated that if platelet fibrinogen is coded by a separate gene or genes (*8*), then amino acid differences would likely be found in these regions if they exist at all. We also compared platelet and plasma fibrinogens by a variety of other means, including SDS-gels with and without reduction to subunits (*9*), presence of carbohydrate in the subunit chains (*10*), clotting and clot-stabilization, and fibrin amino terminal analysis. By all these criteria the bulk of fibrinogen isolated from human platelets was indistinguishable from plasma fibrinogen. On the other hand, platelet fibrinogen as isolated tends to be somewhat more heterogeneous than plasma fibrinogen, apparently due to attack by degradative enzymes in vivo, during platelet storage, or during the isolation procedure. Some of this breakdown can be retarded by a judicious employment of protease inhibitors (*11*).

Human blood platelets were obtained from the San Diego Blood Bank and were usually processed within 72 hours of collection. Platelets (20 blood bank units) were washed according to the procedure outlined by Ganguly (*2*), suspended in a buffer consisting of 0.1*M* tris-HCl, 0.1*M* NaCl, and 0.001*M* ethylenediaminetetraacetate (EDTA) (*p*H 7.2) containing 0.01*M* tosyl-L-arginyl sarcosine methylester (TASME) and 0.01*M* ε-aminocaproic acid (EACA). The suspension was subjected to sonification by three 30-second blasts on a Branson Sonifier and then centrifuged at 27,000*g* for 30 minutes. The supernatants were fractionated by the addition of saturated ammonium sulfate to a concentration of 40 percent saturation, the resulting precipitate was harvested, redissolved in the buffer consisting of tris, NaCl, EDTA, TASME, and EACA, and reprecipitated by the addition of saturated ammonium sulfate to a final concentration of 33 percent saturation. This second precipitate was redissolved in a mixture of 0.1*M* tris-HCl, 0.1*M* NaCl, and 0.001*M* EDTA (*p*H 7.2) and was dialyzed against 0.039*M* tris-phosphate buffer (*p*H 8.6) containing 0.001*M* EACA in preparation for chromatography on diethylaminoethyl (DEAE)-cellulose. During the dialysis the major contaminating protein (thrombasthenin A) precipitates and is readily removed by centrifugation. The supernatant containing clottable protein was applied to a DE-52 column and fractionated with a gradient employing equal volumes of a 0.039*M* tris-phosphate (*p*H 8.6) starting buffer and a 0.5*M* tris-phosphate (*p*H 4.1) limit buffer (*12*). The major peak was highly purified platelet fibrinogen. Pools were concentrated by precipitating with solid ammonium sulfate (0.3 g/ml); they were redissolved in 0.3*M* NaCl–0.005*M* sodium phosphate (*p*H 6.8) and then dialyzed against this same buffer to remove residual ammonium sulfate.

Purified platelet fibrinogen preparations contained more than 80 percent clottable protein (*13*). Sodium dodecyl sulfate–polyacrylamide-gel electrophoresis revealed that the bulk of the platelet fibrinogen was the same size as plasma fibrinogen (Fig. 1). There was some indication that more of the α-chains had been degraded than is usually observed in the plasma fibrinogen case, and this may explain the slightly reduced clottability of these preparations. When gels were stained with the periodic acid–Schiff reagent for carbo-

Table 1. Amino acid compositions of fibrinopeptides isolated from human platelet fibrinogen. Compositions are given as moles per 100 ml as determined after 24 hours of hydrolysis with 5.7*N* HCl at 110°C in sealed tubes under vacuum. Amino acids are listed in the order in which they emerge on the Spinco model 119 amino acid analyzer. The values listed in parentheses are the number of residues present in fibrinopeptides isolated from human plasma fibrinogen. A small amount of cross-contamination between the fibrinopeptides B and A′ is apparent, which is a consequence of their similar electrophoretic mobilities. Platelet fibrinopeptide A′ has an electrophoretic mobility indistinguishable from plasma fibrinopeptide PA (the serine residue of which is phosphorylated) and is probably identical with it.

Amino acids	Fibrinopeptide A	Fibrinopeptide B	Fibrinopeptide A′
Aspartic acid	2.07 (2)	2.84 (3)	2.30 (2)
Threonine			
Serine	0.96 (1)	1.08 (1)	1.01 (1)
Glutamic acid	2.11 (2)	2.85 (3)	2.26 (2)
Proline			
Glycine	4.94 (5)	2.28 (2)	4.25 (5)
Alanine	1.98 (2)	1.17 (1)	1.93 (2)
Cysteine			
Valine	0.96 (1)	1.06 (1)	1.16 (1)
Methionine			
Isoleucine			
Leucine	1.01 (1)	0.20 (0)	0.92 (1)
Tyrosine			
Phenylalanine	1.05 (1)	1.77 (2)	1.23 (1)
Histidine			
Lysine			
Arginine	0.92 (1)	0.94 (1)	0.93 (1)
Total residues	16	14	16

hydrate, the β-chains and γ-chains stained strongly, but α-chains did not, just as in the case of plasma fibrinogen (*14*). Gels run on cross-linked platelet fibrin revealed the formation of γ-γ dimers with mobilities indistinguishable from plasma fibrin controls (Fig. 1), which indicates that γ-chains in these preparations had maintained their integrity completely and that their carboxy terminals must be intact (*15*).

Quantitative amino terminal analysis of platelet fibrin by a thioacetylation procedure (*16*) yielded 2 moles of glycine for every mole of tyrosine, just as in the case of fibrin derived from plasma fibrinogen (*17*). The yields (about 60 percent when calculated on a half-molecule molecular weight of 170,000) were comparable to amino terminal determinations performed on many different fibrin preparations analyzed in the course of other experiments. No spurious amino terminals were observed.

Fibrinopeptides were isolated from platelet fibrin preparations by means of previously described procedures (*18*). The amino acid compositions of the fibrinopeptides A and B isolated from platelet fibrinogen were identical to the known compositions of the fibrinopeptides isolated from human plasma fibrinogen (Table 1). Moreover, the electrophoretic patterns were indistinguishable from the plasma fibrinogen type, including a derivative fibrinopeptide A which has a greater anodal mobility than the major A peptide and corresponding to an A peptide known to be phosphorylated in the case of plasma peptides. Although we did not analyze directly for phosphate, it is likely that this peptide (designated A′ in Table 1) is identical with plasma fibrinopeptide PA (*19*).

It has been claimed that fibrinogen can be synthesized in platelets (*20*) and that no exchange occurs between the plasma type—synthesized in the liver—and the platelet variety (*21*). In persons with afibrinogenemia, levels of immunologically cross-reacting material (fibrinogen) are greatly reduced, although the fact that any is found at all has been interpreted as support for separate pools (*22*). In contrast, our data are most consistent with platelet fibrinogen being synthesized by the same gene or genes as liver-synthesized material, wherever the actual site of synthesis. Moreover, post-translational modifications are apparently the same in the two situations, including the distribution of carbohydrate on subunits and the existence of a phosphorylated serine in the fibrinopeptide A.

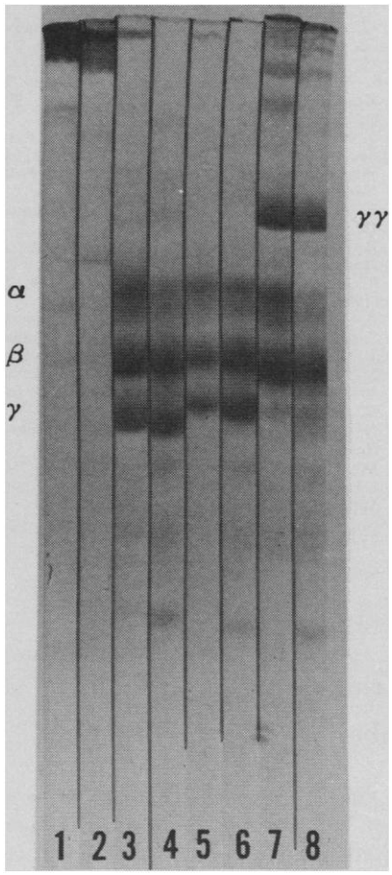

Fig. 1. Sodium dodecyl sulfate–gel electrophoresis (6 percent polyacrylamide) of human plasma and platelet fibrinogens and fibrins: 1, plasma fibrinogen, unreduced; 2, platelet fibrinogen, unreduced; 3, plasma fibrinogen, reduced; 4, platelet fibrinogen, reduced; 5, fibrin (reduced) prepared from plasma fibrinogen; 6, fibrin (reduced) prepared from platelet fibrinogen, 7, cross-linked fibrin (reduced) prepared from plasma fibrinogen; 8, cross-linked fibrin (reduced) prepared from platelet fibrinogen.

Some other proteins found in platelets are known to be identical gene products with their plasma counterparts, including albumin (*23*) and the a-chain of factor XIII (*24*). On the other hand, some plasma proteins (including the b-chain of factor XIII) are not found in platelets, and many platelet proteins (for example, thrombasthenin) are not found in plasma. No clear pattern is yet evident as to which proteins are included or excluded, either on a functional or mechanistic basis, although the fact that platelet fibrinogen is greatly reduced in persons afflicted with thrombasthenia (*22*) might suggest that fibrinogen accumulates in platelets as a direct consequence of binding to thrombasthenin. The kinetics of this interaction could easily be of the sort whereby the phenomenon would not have been detected by simple exchange studies (*21*).

In summary, platelet and plasma fibrinogens appear to be identical gene products, although platelet fibrinogen is somewhat more heterogeneous than the plasma type because of proteolytic abuse, either in in vivo, during platelet storage, or during the isolation procedure. The behavior of these partially degraded molecules follows the same trends as those observed to a lesser degree by plasma fibrinogen, however. In this regard, it has been observed that the α-chains of fibrinogen are exceptionally vulnerable to proteolytic attack and that circulating plasma fibrinogen is a heterogeneous population as a result (*25*). Moreover, the degraded plasma molecules tend to be more soluble and less clottable than their undegraded counterparts (*26*). It is our contention that most previous investigators have been working with platelet fibrinogen preparations that have contained disproportionate amounts of these degraded components or have been less well purified, or both, thereby accounting for the reported differences between plasma and platelet fibrinogens.

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R. F. Doolittle, *Biochim. Biophys. Acta* **258**, 577 (1972)] and e-aminocaproic acid instead of Trasylol and glutamyl-tyrosine—sonicated instead of homogenizing, and shortened the procedure by at least one column step by eliminating gel filtration on G-200. We also found that the major contaminant in the preparation was precipitated in the overnight dialysis before DEAE-cellulose chromatography.
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Biological Suppression of Weeds: Evidence for Allelopathy in Accessions of Cucumber

Abstract. *Cucumber* (Cucumis sativus *L.*) *accessions from 41 nations were grown with two indicator species in a search for superior competitors. Of the plant introductions tested, one inhibited indicator plant growth by 87 percent and 25 inhibited growth by 50 percent or more. The toxicity of leachates from pots containing inhibitory cucumbers to indicator plants germinated in separate containers suggested allelopathy. Incorporation of an allelopathic character into a crop cultivar could provide the plant with a means of gaining a competitive advantage over certain weeds.*

Successful biological control of weeds is limited to a few instances where introduced predators have reduced populations of a target species. No biological methods have gained wide acceptance for selective use in agronomic crops (*1*). Although plant breeders have successfully incorporated both insect and disease resistance into cultivars of many crops, there has been no concerted effort to develop crops with superior competitive ability with weeds.

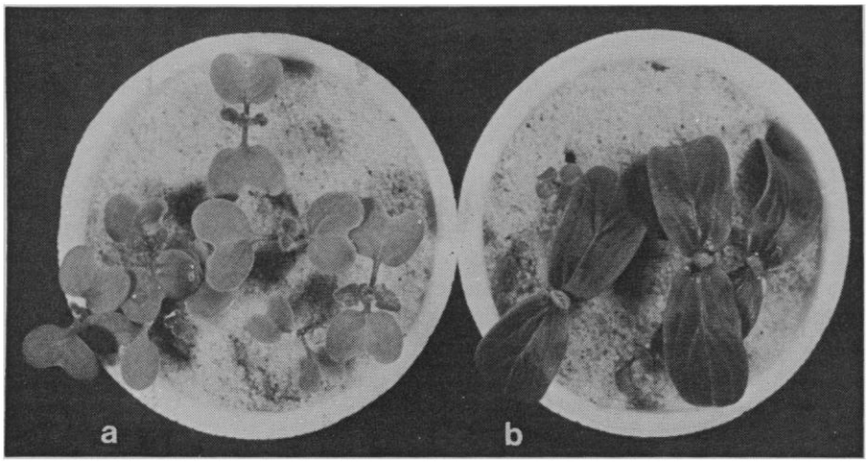

Fig. 1. Growth of *Brassica hirta* after 7 days (a) in the absence of cucumber and (b) in the presence of plant introduction 163221. Ten indicator seeds were planted in each pot.

Differences in competitive ability have been reported among cultivars or inbred lines of *Sorghum bicolor* (L.) Moench (*2*), *Oryza sativa* L. (*3*), *Triticum aestivum* L. (*4*), and *Glycine max* (L.) Merr. (*5*) exposed to various weed species. In these instances, competitive advantage was attributed to rapid growth and an outstanding ability to compete for light, water, or nutrients.

Allelopathy, the inhibition of growth of one species by chemicals released from another, occurs widely in natural plant communities (*6*, *7*) and may regulate the density and distribution of species. Certain crop plants, including those in the genera *Avena*, *Triticum*, and *Secale* (*8*); *Hordeum* (*9*); *Bromus* (*10*); *Brassica* (*6*); and *Nicotiana* (*11*), are known to release toxic substances from their roots. The release of allelopathic chemicals may occur by exudation from living roots or may occur upon their death and decay. The only way man has utilized allelopathy to his agronomic advantage is by planting crops such as *Hordeum vulgare* L. as interim "smoother crops" to reduce weed infestations (*9*).

We hypothesized that predecessors of many species now grown for food or fiber, when growing in their wild habitat, may have possessed allelopathic substances which allowed them to compete effectively in their native plant community. This character may have been reduced or lost as plants were bred and selected for other desirable characteristics in a weed-free environment. Furthermore, resistance to insects and diseases has been incorporated into several crops by crossing with "wild types." We chose cucumber as a test species because it has considerable genetic diversity, its tolerance to effective herbicides is less than adequate, and it may be grown at high plant populations which would favor the success of allelopathy in the field.

We obtained the collection of cucumber seed from the U.S. Department of Agriculture, North Central Introduction Station, Ames, Iowa. The collection consisted of 526 accessions of *Cucumis sativus* L. and 12 accessions of eight related *Cucumis* species representing 41 nations of origin. We also included four commercial cultivars as reference standards. In the initial, unreplicated study, we planted four seeds of each accession with ten seeds of each indicator species in 7.62-cm styrofoam pots containing 270 g of quartz sand. As indicators, we chose a monocot (*Panicum miliaceum* L.) and a dicot