

Interactions of Various Homopolypeptides with Human Platelet-Rich Plasma Suspensions

BARTH H. RAGATZ

Fort Wayne Center for Medical Education,
Indiana University School of Medicine,
Fort Wayne, Indiana 46805

Introduction

A number of reports indicate that homogeneous polymers of L-lysine of various molecular weights will induce aggregation in stirred suspensions of human platelet-rich plasma (PRP) (2, 3, 4, 6, 7, 12). This aggregation phenomenon is concentration dependent (4, 7), molecular weight (size) independent in the range 2,800-194,000 daltons (6, 12), positive charge dependent (5, 7), and is blocked by certain naturally occurring polymers (heparin, chondroitin sulfate) with net negative charge (2, 7). There are conflicting reports as to whether the aggregation is calcium dependent (3, 4), plasma cofactor dependent (4), and whether it can be blocked by inhibitors of ADP induced aggregation or inhibitors of the platelet release reaction (2, 3).

The goals of this project are to expand the understanding of the polymer-platelet interaction at these several levels: (a) What are the interactions of other polymers (cationic, anionic) with PRP? (b) Are there smaller molecules (similar in monomer conformation) which can competitively saturate polymer receptor sites? (c) Are there cooperative interactions between polymer receptors and other receptors on the platelet surface?

Materials and Methods

Freshly collected whole blood from normal medication-free human subjects are rapidly mixed with one-tenth volume of 3.2% sodium citrate. Platelet-rich plasma (PRP) was prepared by differential centrifugation and the platelet count was adjusted to approximately $400,000/\text{mm}^3$ by dilution with 0.155 M NaCl. All homopolypeptides were obtained from either Sigma Chemical Company (St. Louis, Missouri) or from Miles Laboratories (Elkhart, Indiana) and were diluted in 0.155 M NaCl and adjusted to pH 7.0. Polymers utilized include Miles low molecular weight (MW = 14,000) poly-L-lysine (LPL); Sigma high molecular weight (MW = 165,000) poly-L-lysine (HPL); Sigma poly-L-arginine (MW = 14,000) (PA); Sigma poly-L-histidine (MW = 15,000) (PH); Sigma poly-L-glutamic acid (MW = 38,000) (PG); and Sigma poly-L-tyrosine (MW = 78,000) (PT). Platelet aggregation was studied by the turbidimetric method of Born (1) in a Payton dual channel aggregometer. Samples of PRP (0.5 ml) were placed in cuvettes within the aggregometer and were stimulated after 30 seconds by addition of 0.05 ml polypeptide solutions. In other experiments, 0.5 ml PRP was incubated initially with 0.05 ml of Sigma L-lysine or Sigma L-arginine, and challenged with an appropriate polymer (0.05 ml) 2-5 minutes later. Finally, some PRP samples (0.5 ml) were incubated with LPL (0.05 ml) initially and stimulated by Sigma adenosine - 5' - diphosphate (ADP) additions (0.05 ml) from 30 seconds to 6 minutes later.

Results

A typical example of platelet aggregation induced by a poly-L-lysine preparation is shown in Figure 1. The extent of aggregation is seen to be polymer concen-

- A. HPL (0.01mg/ml)
B. HPL (0.1mg/ml)
C. HPL (1.0mg/ml)

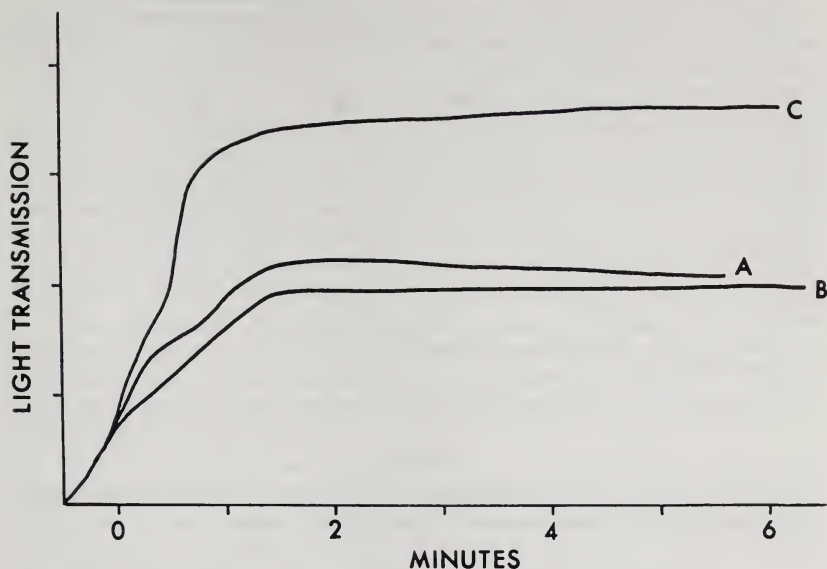


FIGURE 1: *Effect of additions of various concentrations of high molecular weight poly-L-lysine to stirred human platelet-rich plasma.*

tration dependent over a 100-fold concentration range. Similar concentration dependent results were obtained also for LPL over a 10-fold concentration range (0.1 mg/ml-1.0 mg/ml). Both experiments are in agreement with published data for poly-L-lysine preparations obtained in other labs (4, 7).

Under similar conditions, the strongly cationic polypeptide, PA, induced a vigorous aggregation, while the weakly cationic polypeptide, PH, was without effect on PRP for incubation periods up to 7 minutes. The strongly anionic polypeptide, PG, and the weakly anionic polypeptide, PT, were both devoid of inducing aggregation or producing shape changes in platelets during incubation periods of 5-7 minutes

Figure 2 shows that incubation of PRP with L-arginine causes no measurable turbidimetric change. Furthermore, if PA is added after incubating PRP with L-arginine for 3 minutes, the PRP sample is almost completely aggregated without any delay period. Analogous results have been obtained using PRP plus L-lysine, and PRP plus L-lysine plus HPL combinations.

At this point in time, data have been obtained which indicate that LPL and ADP interact together in an additive manner in bringing about platelet aggregation. Figure 3 shows that PRP incubated with a suboptimal concentration of LPL can be stimulated to aggregate additionally if ADP is added 30 seconds to 6 minutes later.

A. PA (1.0mg/ml)

B. L-Arginine(1.0mg/ml)+PA(1.0mg/ml) 3' Later

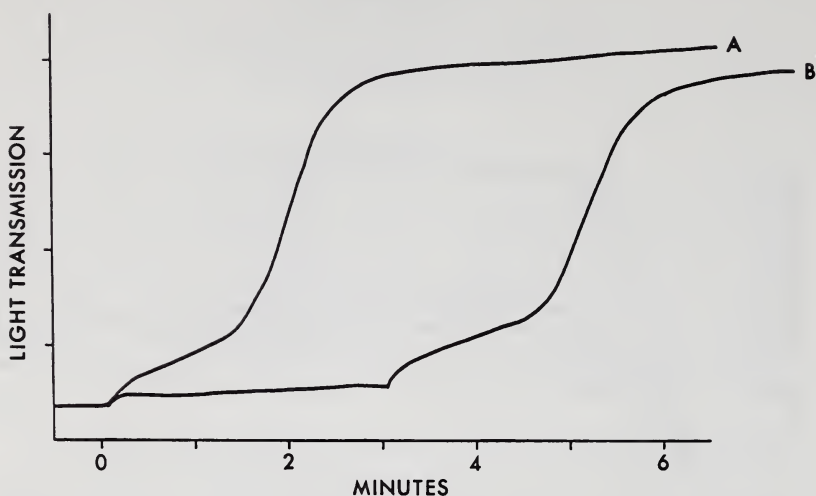


FIGURE 2: Examination of L-arginine as a potential competitive inhibitor of poly-L-arginine induced aggregation of human platelet-rich plasma.

A. LPL (0.1mg/ml)+ADP(1×10^{-4} M) 30" Later

B. LPL (0.1mg/ml)+ADP(1×10^{-4} M) 2' Later

C. LPL (0.1mg/ml)+ADP(1×10^{-4} M) 6' Later

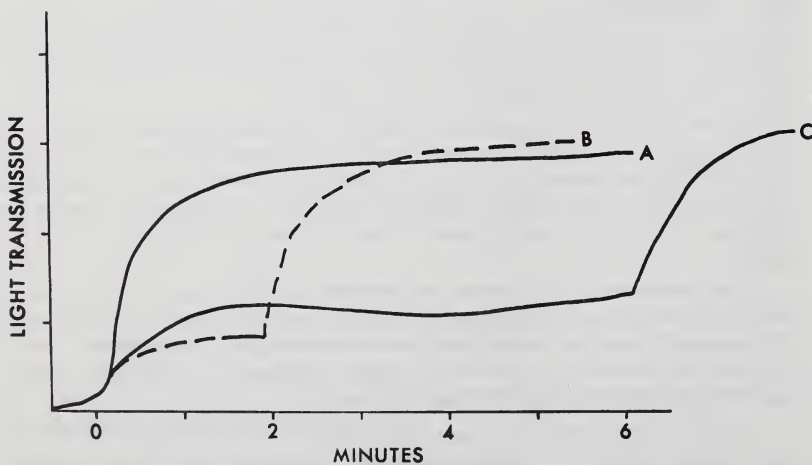


FIGURE 3: Effect of time dependent additions of adenosine-5'-diphosphate to stirred human platelet-rich plasma suspensions pretreated with low molecular weight poly-L-lysine.

Discussion

Present results for poly-L-lysine polymers are in agreement with those of other investigators; namely, that polymers in the molecular weight range 14,000 - 165,000 are effective in inducing a dose dependent aggregation of PRP. HPL seemed to be more effective at lower concentrations than LPL in producing aggregation. Literature reports suggest that poly-L-lysine samples as small as molecular weight 2,500 - 4,000 are able to stimulate platelet aggregation (4, 6, 7, 12). A low molecular weight poly-L-arginine, PA (MW = 14,000) is able to stimulate aggregation, but a poly-L-histidine sample, PH, of similar molecular size (MW = 15,000) is devoid of effect. The anionic polymers, PG and PT, are also both without effect on PRP. These results obtained for five kinds of polymers suggest that the proaggregatory effect is limited to poly cations only; hence, it is probably caused by electrostatic interaction between polymer and platelet surface, rather than by action at a receptor. That PA is stimulatory and PH is not suggest that charge density and steric factors have some importance in determining the platelet-polymer interaction and that monomer structure is not a specific determinant.

The importance of significant positive charge density on poly-L-lysine samples is supported also by the observation that succinylation of the terminal epsilon amino groups of such polymers abolishes proaggregatory activity (7). Similar abolition of activity occurs after D-galactose is covalently coupled with poly-L-lysine epsilon amino groups (5).

That the interaction between platelets and certain polycations is principally electrostatic is also supported by the following literature reports: (a) Natural polyanions (heparin, chondroitin sulfates) form complexes with polylysine and block the polymer aggregating effect (2, 7) and (b) Even D- or D, L- polylysine samples induce aggregation suggesting that polymer conformation is not an important determinant of functionality (4).

Present results also indicate that large concentrations of either L-lysine or L-arginine are without direct effect on platelets, and these amino acids are unable to occupy any discrete cationic binding sites on platelet membrane. Thus, the amino acids are unable to abolish the effect of either poly-L-lysine or poly-L-arginine. It would not be expected that the initial monomer concentrations added would be significantly lowered by metabolic events taking place in the platelet suspensions within the incubation periods used during these experiments. Jenkins *et al.* previously reported that L-lysine was without direct effect on PRP and had no influence on poly-L-lysine induced aggregation (2). Another literature report, however, related to the effect with added L-arginine stated that large concentrations of this amino acid inhibited collagen cross linking and multimer formation, rendering the fibrous protein inactive for aggregation of platelets (11). The lack of binding or interaction of basic amino acids with platelet membrane was also extended by Rennert *et al.* who reported that L-arginine and the urea cycle intermediate, L-ornithine, were both without effect on platelet aggregation induced by ADP, epinephrine, or ristocetin (9).

At present, our experiments have shown a cooperative interaction between LPL and ADP in induction of platelet aggregation. Massini *et al.* also observed that ADP added to PRP 30 seconds after addition of poly-L-lysine (MW = 50,000) enhanced aggregation (4). These effects may be easier to observe with smaller polymers as used in the present study or in the Massini *et al.* report since large molecular weight polymers may obstruct access of ADP or other inducers to specific platelet receptor sites. As seen in Figure 3 from the similar slopes and max-

ima of the various curves, the interaction of ADP and LPL with platelets appears to be additive. These results are compatible with the hypothesis that cationic polymers attract platelets into close proximity by electrostatic interactions and that ADP acting at a specific unmasked receptor stimulates platelet aggregation and release of stored aggregating substances.

Polycations have been shown to have other biological effects and additional interactions with plasma proteins which may be of importance to future studies with PRP. Vogel *et. al.* have used poly-L-lysines (MW range 6,900-72,000) to accelerate conversion of prothrombin to active thrombin by activated clotting factor X (13). Nossel *et. al.* reported that poly-L-lysine samples (MW range 4,000 - 100,000) inhibit the surface activation of Hageman factor (factor XII) (8). Secondary interactions of platelets with proteins of the blood coagulation or fibrinolysis systems can be eliminated by conducting experiments with platelets suspensions washed free of plasma proteins. Finally, Ryser, reported that poly-L-ornithine samples (MW range 45,000 - 200,000) or poly-L-lysine samples (MW range 3,000 - 225,000) increase cell permeability (measured by albumin uptake) in Sarcoma S-180 cell cultures (10). It is presently unknown if platelet permeability is modified by polycations.

Future experiments in this lab will be directed at investigation of potential interactions of small cationic polymers with other platelet aggregation inducer receptor sites, e.g., epinephrine receptors. Small cationic agents which can serve as antagonists that will bind to platelet surface without producing aggregation and will block aggregatory effects of larger polymers will be sought. The potential clinical relevance of these experiments is obvious in view of the undesirable interactions between platelets and biological polymers of damaged arterial wall or synthetic polymers found in certain prosthetic heart valves.

Acknowledgments

The author wishes to acknowledge the encouragement and financial support of Dr. P. G. Iatridis for the project while the author was a faculty member of the Northwest Center for Medical Education, Gary, Indiana.

Literature Cited

1. BORN, G. V. R. 1962. Aggregation of Blood Platelets by Adenosine Diphosphate and Its Reversal. *Nature*. 194:927.
2. JENKINS, C. S. P., M. A. PACKHAM, R. L. KINLOUGH-RATHBONE and J. F. MUSTARD. 1971. Interactions of Polylysine with Platelets. *Blood*. 37:395-412.
3. MASSINI, P. and E. F. LUSCHER. 1972. On the Mechanism by which Cell Contact Induces the Release Reaction of Blood Platelets; the Effect of Cationic Polymers. *Thrombosis*. 27:121-133.
4. MASSINI, P., L. C. METCALF, U. NAF and E. F. LUSCHER. 1974. Induction of Aggregation and of the Release Reaction in Human Platelets by Polylysine. *Haemostasis*. 3:8-19.
5. MESTER, M., B. KRASKA, J. CRISBA and L. MESTER. 1976. Effect of Amadori-Type Sugar Derivatives of Poly-L-lysine on Platelet Aggregation. *Haemostasis*. 5:115-119.
6. METCALF, L. C. and D. J. LYMAN. 1974. The effect of Conformational Changes on the Blood Platelet Reactivity of Polylysine. *Thrombosis Research*. 5:709-717.
7. MOHAMMAD, S. F., H. Y. K. CHUANG and R. G. MASON. 1977. Roles of Polymer Size and ϵ -Amino Groups in Polylysine-Platelet Interaction. *Thrombosis Research*. 1:193-202.
8. NOSSEL, H. L., H. RUBIN, M. DRILLINGS and R. HSIEH. 1968. Inhibition of Hageman Factor Activation. *J. Clin. Invest.* 47:1172-1180.

9. RENNERT, O., B. BUEHLER, T. MIALE and D. LAWSON. 1976. Polyamines and Platelet Aggregation. *Life Sciences*. 19:257-264.
10. RYSER, H. J. P. 1967. A Membrane Effect of Basic Polymers dependent on Molecular Size. *Nature*. 215:934-936.
11. SIMONS, E. R. , C. M. CHESNEY, R. W. COLMAN, E. HARPER and E. SAMBERG. 1975. The Effect of the Conformation of Collagen on its Ability to Aggregate Platelets. *Thrombosis Research*. 7:123-139.
12. TIFFANY, M. L. and J. A. PENNER. 1976. Polylysine Aggregation of Human Blood Platelets. *Thrombosis Research*. 8:529-530.
13. VOGEL, C. N., R. J. BUTKOWSKI, K. G. MANN and R. L. LUNDBLAD. 1976. Effect of Polylysine on the Activation of Prothrombin. Polylysine Substitutes for Calcium Ions and Factor V in the Factor Xa Catalyzed Activation of Prothrombin. *Biochem*. 15:3265-3269.