(*18, 19*), (ii) a change in the state of protonation of the Schiff base (*20*), and (iii) a molecular rearrangement in the protein induced by electron redistribution in the chromophore (*21*).

Isomerization is an unlikely candidate in our case. Not only is the bacteriorhodopsin chromophore initially all *trans*, but 13-*cis* is the only other isomer that can combine with the bacteriorhodopsin protein matrix, and it leads to a blue shift of the absorption maximum relative to bR_{570} (*22*). Time-resolved measurements also indicate that such molecular rearrangements occur on a slower time scale (*23, 24*).

The second suggestion can be discounted on the basis of experiments by Marcus and Lewis (*25*). With kinetic resonance Raman studies they demonstrated that the state of protonation of the Schiff base changes on a time scale of 10 to 30 μsec following photoexcitation. This is certainly too slow for the intermediate formation we observed.

Our results are consistent with the third possibility, proposed by Lewis (*21*). In this proposal, light absorption results in a significant electron redistribution in the retinylidene chromophore (*26*). This, in turn, induces a protein conformational transition. One possibility might be proton movement from one amino acid to another. Such a rearrangement could introduce the appropriate absorption changes (*26, 27*), would be sensitive to deuteration (*20*), and could also excite the proton translocating function of this biological light-driven proton pump.

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Thrombin-Induced Platelet Aggregation Is Mediated by a Platelet Plasma Membrane-Bound Lectin

Abstract. *Thrombin-activated human platelets cause agglutination of trypsinized, formalinized bovine erythrocytes. This lectin activity of stimulated platelets was blocked by galactosamine, glucosamine, mannosamine, lysine, and arginine, but not by N-acetylated sugars, other neutral sugars, or other amino acids. Inhibitors of the thrombin-induced lectin activity also blocked thrombin-induced platelet aggregation. It appears that a membrane surface component that has lectin activity mediates platelet aggregation.*

The mechanisms underlying membrane-membrane interaction are important for understanding many problems that are central to cell and developmental biology, such as differentiation, cell aggregation, viral infectivity, and fertilization. The systems that have been useful in identifying membrane components responsible for direct cell-cell interaction involve experimental manipulation of the membrane surface. Starvation of slime mold amoebas causes an alteration of the membrane surface with a concomitant increase in cell-cell interaction (*1, 2*); similarly, differentiation of myoblasts appears to alter the cell membrane to facilitate cell fusion (*3, 4*). In these examples, the membrane surface components implicated in cell-cell interaction have lectin activity; that is, they can agglutinate trypsinized, formalinized erythrocytes. In the lectin model of cell-cell interaction (*5*), cell contact is mediated by a lectin molecule on one cell that specifically attaches to receptors on an adjacent cell.

Platelet aggregation is also a process that involves interaction of membrane surfaces. This process causes the primary arrest of blood flow (hemostasis) and the formation of arterial thrombi (thrombosis). The most potent physiological agent for inducing platelet aggregation is thrombin. Thrombin activation of platelets causes a change in platelet shape and an alteration in the surface membrane, resulting in the platelets becoming cohesive and aggregated (*6*).

We have previously reported that platelet plasma membranes have lectin activity (*7*). We now report that thrombin stimulation of intact cells causes the expression of a lectin activity not seen with unstimulated cells. This activity appears to mediate at least the initial stages of platelet aggregation.

The lectin activity of intact, washed human platelets was measured by their ability to agglutinate trypsinized, formalinized erythrocytes from different species. The erythrocytes had no lectin activity, but as the source of receptors could be cross-linked by appropriate lectins. In this assay, washed platelets agglutinated erythrocytes from species such as cow, electric eel, rabbit, catfish, and sheep.

After stimulation by the thrombin, platelets had enhanced lectin activity (Fig. 1). Unlike the activity of unstimulated platelets that caused the agglutination of erythrocytes from many species, the enhanced lectin activity was detected only with cow or sheep erythrocytes and not with rabbit erythrocytes as a source of receptor. This erythrocyte species specificity is similar to that seen in the lectin activity of isolated platelet

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membranes (*7*). Thus, cell disruption, or thrombin stimulation, is sufficient to cause the expression of a lectin activity not present on the surface of unstimulated platelets.

Two inhibitors of platelet aggregation, cytochalasin B (*8*) and prostaglandin E_1 (*9*), when used at concentrations that inhibited platelet activation, prevented the expression of enhanced lectin activity (Fig. 1). Since cytochalasin B and prostaglandin E_1 have no effect on platelet-thrombin interaction (*10*), it suggested that the thrombin-enhanced lectin activity was not the result of thrombin cross-linking of cells. Thrombin itself had no lectin activity against the erythrocytes tested. Thus, platelet stimulation, rather than thrombin cross-linking of cells, appeared to cause the enhanced lectin activity.

Thrombin-platelet interaction causes a change in the selective secretion of several types of macromolecules (*11*). However, the enhanced lectin activity of activated platelets was not caused by these released materials; the supernatant from thrombin-treated platelets did not have lectin activity, nor did it enhance the lectin activity of platelets inactivated by cytochalasin B. In addition, scanning electron micrographs of erythrocytes agglutinated by activated platelets revealed only platelet-erythrocyte interaction, not erythrocyte-erythrocyte interaction (Fig. 2).

Trypsin, at a concentration that did not cause platelet lysis, inhibited the lectin activity of both stimulated and unstimulated platelets. This suggested that the enhanced activity was caused by expression of a protein on the surface of stimulated platelets.

The specificity of lectin activity associated with cell surfaces can be determined by competition between certain compounds and erythrocyte receptors for the platelet membrane-bound lectins (*2–4*, *12*). A variety of sugars and amino acids were tested for inhibition of the thrombin-enhanced lectin activity. The enhanced lectin activity was blocked by 30 m*M* of the amino sugars galactosamine, glucosamine, and mannosamine, and the basic amino acids, lysine and arginine; however, none of these compounds inhibited lectin activity of unstimulated platelets (Fig. 1). Of the three amino sugars tested, mannosamine was the most effective inhibitor. Neutral sugars, *N*-acetylated amino sugars, and other amino acids had no effect on either activity (*13*). The inhibition was not caused by an alteration in ionic strength since 30 m*M* glutamine, asparagine, and ammonium chloride did not inhibit. It appeared that the inhibitor required a charged amino group.

Thrombin stimulation of platelets causes an alteration in the membrane surface that results in the parallel expression of enhanced lectin activity and membrane cohesiveness. To test whether the same proteins mediate both functions, we examined the possibility that the substances that block thrombin-induced lectin activity may also block thrombin-induced platelet aggregation. The same amino sugars and basic amino acids (*14*) that blocked the enhanced lectin activity also blocked thrombin-induced platelet aggregation (Fig. 3); the compounds that failed to inhibit thrombin-induced lectin activity had no effect on thrombin-induced aggregation.

The lectin model for cellular aggregation would predict that inhibition of thrombin-induced aggregation by amino

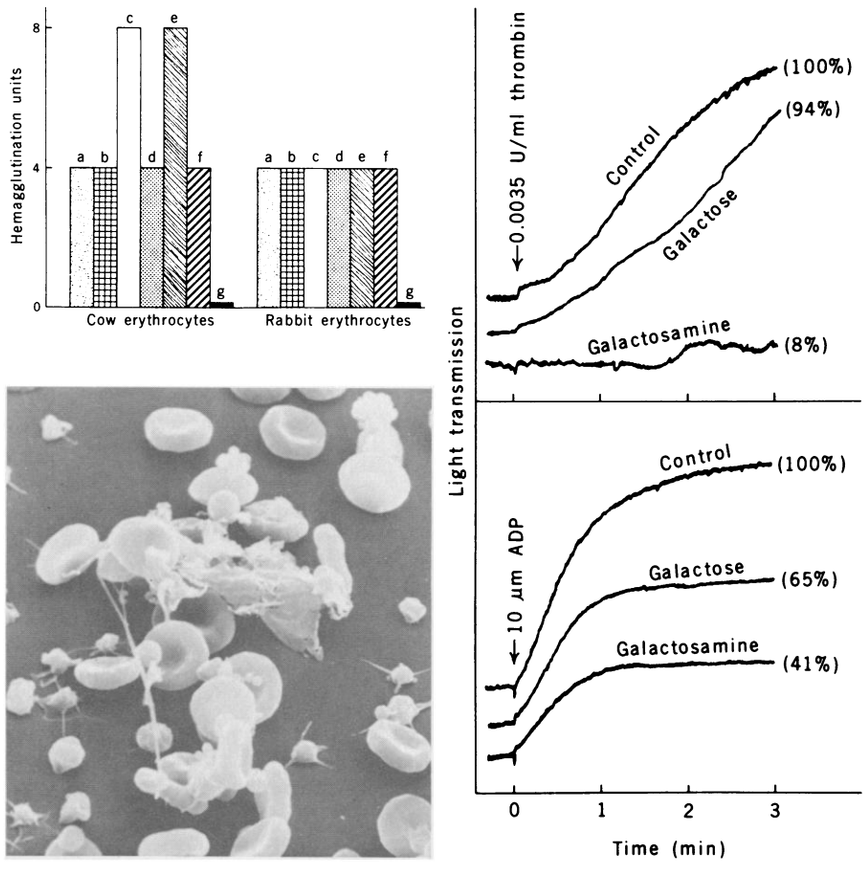

Fig. 1 (top left). Platelet lectin activity of washed platelets or platelets treated with thrombin (thrombin-stimulated platelets), mixed with erythrocytes from the species indicated; hemagglutination activity was determined (*7*): *a*, washed platelets in the absence of added inhibitors; *b*, washed platelets in the presence of 30 m*M* galactosamine or arginine; *c*, thrombin-stimulated platelets in the absence of added inhibitors; *d*, thrombin-stimulated platelets in the presence of 30 m*M* galactosamine or arginine; *e*, thrombin-stimulated platelets in the presence of 30 m*M* galactose, *N*-acetylgalactosamine, or glutamine; *f*, washed platelets incubated with prostaglandin E_1 (10 μM) or cytochalasin B (5 μg/ml) for 1 hour before exposure to thrombin (an identical titer was obtained when platelets were incubated with either reagent and subsequently stimulated with thrombin); and *g*, thrombin-stimulated platelets treated for 15 minutes with trypsin (25 μg/ml) at 22°C. The data presented are the results from one platelet preparation; each titer represents data from quadruplicate samples. Other platelet preparations yielded similar data. Fig. 2 (bottom left). Thrombin-stimulated washed human platelets (10 μl of a 1×10^9 platelets per milliliter suspension) were mixed with 10 μl of a 2.5 percent suspension of trypsinized, formalinized bovine erythrocytes in 25 μl phosphate-buffered saline and allowed to settle on a glass cover slip for 45 minutes. For scanning electron microscopy, the cells were fixed with 4 percent glutaraldehyde in Millonigs phosphate buffer (*15*), dehydrated in an ascending series of ethanol, critically point dried (*16*), mounted on stubs, and coated with a layer of gold-palladium. Fig. 3 (right). Effect of galactose and galactosamine on thrombin- and ADP-induced aggregation. Washed platelets at 10^9 per milliliter in Tyrode's solution were placed in an aggregometer cuvette with the indicated concentrations of stimuli, either bovine thrombin (1800 units of protein per milligram) or ADP (Sigma) containing 50 μg of human fibrinogen per milliliter (Kabi) was added. Galactose or galactosamine, dissolved in Tyrode's solution and adjusted to *p*H 7.4, was added at a final concentration of 0.015*M* before the addition of the stimulus. No addition had an effect on the final *p*H after aggregation. Light transmission was continually monitored (*17*). Values in parentheses represent the percentage of platelet aggregation.

sugars and amino acids is caused by binding of inhibitors to the active sites of the lectins. Alternatively, those inhibitors could prevent thrombin activation and, thereby, block the exposure of a cryptic form of the surface protein that mediates aggregation. This alternative appears unlikely, however, since (i) the aggregation induced by adenosine diphosphate (ADP), a nonproteolytic stimulus, was also inhibited by the amino sugars (Fig. 3); (ii) the amino sugars and basic amino acids caused no more than a 20 percent inhibition of thrombin-induced serotonin secretion (data not shown); and (iii) inhibition of the thrombin-enhanced lectin activity was observed after platelet activation had occurred. Therefore, we conclude that the amino sugars and basic amino acids did not block platelet aggregation by preventing platelet activation. Although neutral sugars had no effect on thrombin-induced aggregation, galactose (Fig. 3) had a slight effect on ADP-induced aggregation. This difference may reflect some inherent difference between ADP- and some thrombin-induced aggregation of platelets.

The data presented here demonstrate that platelet activation causes the expression of a previously inactive lectin that appears to mediate at least the initial phases of platelet aggregation by specifically binding to receptors on adjacent platelets. Since aggregation of thrombin-stimulated platelets can be blocked by both amino sugars and basic amino acids, the lectin receptor could be either protein or carbohydrate. Further work is required to determine whether platelet cohesiveness is derived entirely from the expression of platelet lectin activity, or whether activation also affects the expression of lectin receptors.

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Antibodies to Purified Insulin Receptor Have Insulin-Like Activity

Abstract. *Antibodies to insulin receptors purified from rat liver membranes do not complete with [^{125}I]insulin for binding to the insulin receptor but do precipitate solubilized receptors labeled with [^{125}I]insulin. These antibodies have the insulin-like activities of enhancing glucose oxidation and inhibiting epinephrine-induced lipolysis in rat adipocytes. Thus, antibody binds to the receptor at a different site from that to which insulin binds, yet the interaction can initiate an effective biological response. These results indicate that the previously studied insulin-binding sites are the physiological macromolecular receptors for insulin.*

Antibodies to the insulin receptor, which compete with insulin for binding to the receptor, occur spontaneously in patients with the Kahn type B syndrome of acanthosis nigricans and insulin resistance (*1*). In three patients with this syndrome, these antibodies have been shown to have insulin-like biological activity (*2*). We now report the production of rabbit antibody to purified insulin receptors that bind to the receptor at a site distinct from that which binds insulin, but can stimulate conversion of glucose to CO_2 and inhibit epinephrine-stimulated lipolysis in isolated fat cells in a manner similar to that of insulin.

Insulin receptor was solubilized from rat liver membranes with 2 percent Triton X-100 and purified by DEAE-cellulose chromatography and affinity chromatography on insulin-agarose and concanavalin A–agarose (*3*, *4*). A rabbit was immunized with this highly purified receptor preparation, which, as revealed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis, contained a major Coomassie blue staining band with an apparent molecular weight of 135,000 and minor bands of lower molecular weight (*4*).

The antiserum to the receptor did not inhibit [^{125}I]insulin binding to rat liver membranes or to rat fat cell membranes, even when high concentrations of antiserum were used (1:5 dilution), or when the membranes were incubated with antiserum for up to 6 hours before [^{125}I]insulin was added (data not shown). A direct interaction between the antiserum to the receptor and the insulin receptor was demonstrated by indirect immunoprecipitation (Fig. 1). Solubilized receptor from rat liver membranes was labeled with [^{125}I]insulin and incubated with antiserum to receptor. The receptor-antibody complex was then precipitated with an antiserum to rabbit immunoglobulin G (IgG) (Fig. 1A). When an excess of native insulin was added to the incubation mixture to prevent receptor labeling, the radioactivity in the precipitate was greatly diminished (Fig. 1B). No [^{125}I]insulin-labeled receptor was precipitated when anti-insulin receptor serum was replaced by serum obtained from the rabbit before immunization or buffer alone (Fig. 1, C and D) or when solubilized receptor was omitted from the incubation mixture (Fig. 1E), a procedure that excluded the possibility that the specific precipitation of [^{125}I]insulin (Fig. 1A) was due to antibodies to insulin rather than to the receptor. The amount of [^{125}I]insulin-labeled receptor precipitated by varying concentrations of antiserum to the receptor was compared with the amount of labeled receptor precipitated by the polyethylene glycol assay (*3*). Both methods resulted in the precipitation of similar amounts of la-

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