Binding of Soluble Type I Collagen to Fibroblasts: Effects of Thermal Activation of Ligand, Ligand Concentration, Pinocytosis, and Cytoskeletal Modifiers

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ABSTRACT Efficient binding of native, soluble ^{125}I-labeled type I rat collagen to mouse 3T3 fibroblast monolayers requires prior warming of the ligand to 35–37°C for 10–30 min. Decreased binding at high ligand concentrations is ascribed to ligand-ligand interactions rather than to negative cooperativity. Addition of bacterial collagenase to monolayers labeled with the ^{125}I-ligand releases a constant fraction (80%) of the bound ligand over a 2-h interval at 37°C, indicating that little of the ligand becomes inaccessible by pinocytosis. Colchicine (10^{-7} M) and vinblastine (5×10^{-8} M) do not inhibit binding by morphologically intact monolayers. Cytochalasins and concanavalin A show dose-related inhibition of binding by intact monolayers that is due to a reduction in the number of available binding sites rather than to a change in binding site affinity. The collagen binding site on the fibroblast surface is proposed as an organizing center for the assembly of periodic type I collagen fibrils.

Collagens, collagen α chains, and collagenous peptides bind to the surface of fibroblasts. Chiang et al. have reported that heat-denatured collagenous molecules bind to the fibroblast surface and induce chemotactic migration of the cells in vitro (1). I have studied the binding of native soluble collagen to the fibroblast and have suggested that polymerization of collagen to form fibrils might be initiated upon the cell surface (2). To gain further insights into functions of surface-bound collagens, I examined in additional detail the binding of native, soluble ^{125}I-labeled type I collagen to fibroblasts. In particular, I analyzed the requirement for thermal activation of the ligand, the effect on binding of ligand concentration, and pinocytosis of the bound ligand. A variety of molecules that could interact with the ligand at the cell surface, alter fibroblast metabolism, or alter cell membrane or cytoskeletal architecture have also been tested for possible effects on collagen binding. These data are interpreted in terms of the proposal that a plasma membrane binding site participates in the initial assembly of collagen fibrils.

MATERIALS AND METHODS

Cells and Binding Assay

The contact-inhibited 3T3 Swiss mouse fibroblast line was cultured in Dulbecco's modification of Eagle's medium (DME) with 10% calf serum. Iodinated lathyritic rat skin collagen (^{125}I-LRSC) was prepared as previously described (2). Freshly prepared ligand stock solutions had specific activities of $\sim 3 \times 10^6$ cpm/μg. For experiments, the ^{125}I-LRSC stock was diluted with binding buffer (DME with half bicarbonate concentration, 0.05 M HEPES, pH 7.2 and 2.5 mg/ml bovine serum albumin) to ~60,000 cpm/ml and warmed at 37°C for 30 min. Confluent 3T3 monolayers in 60-mm plastic petri dishes ($\sim 2 \times 10^6$ cells) were washed three times with 4 ml of binding buffer before receiving 2 ml of the heat-activated ^{125}I-LRSC solution that had been cooled to room temperature (26°C). Binding studies were generally performed at the latter temperature. Rapid washing of the cultures and measurement of radioactivity specifically bound to the cells were performed as described (2).

Protease Digestions: Probes for Triple-Helicity of Collagen

Native soluble unlabeled LRSC, or ^{14}C-(proline and glycine)-labeled lathyritic type I mouse collagen, was incubated at 37°C for 30 min ("thermal activation"), or kept at 4°C (controls) before incubation with proteases. Three digestion protocols were used. (*a*) Digestion with pepsin (Worthington Biochemical Corp., Freehold, NJ; Porcine A), 100 μg/ml in 0.5 M acetic acid. Enzyme/substrate ratio 1/10. Incubation at 15°C for 5 h. Reaction stopped with cooling, addition of pepstatin, and NaOH. (*b*) Digestion with α-chymotrypsin (Worthington Biochemical Corp.; CDI), according to Bornstein et al. (3). Enzyme/substrate ratio 1/10. Incubation at 15°C for 24 h. Reaction stopped by cooling, acidification, addition of leupeptin and pepstatin, and salt-precipitation of the collagen. (*c*) Digestion with mixture of Trypsin (Worthington Biochemical Corp.; TRTPCK) and α-chymotrypsin, according to Bruckner and Prockop (4). Trypsin: 10-fold molar excess; chymotrypsin: 100-fold molar excess; relative to substrate. Incubation at 20°C for 3–15 min. Reaction stopped by cooling, addition of soybean trypsin inhibitor (Sigma Chemical Co., St. Louis, MO), L-(1-tosylamido-2-phenyl)ethyl chloromethyl ketone (Sigma Chemical Co.; TPCK), urea, SDS, and heating at 100°C for 3 min.

The products of digestion were analyzed by electrophoresis in SDS polyacryl-

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amide slab gels. Proteins were visualized by Coomassie Blue staining or fluorography. Resistance to proteolysis was assessed by the relative recoveries of intact helical α chains from experimental and control digests.

Materials

Lathyritic rat skin collagen was donated by Dr. George Martin (National Institute of Dental Research, Bethesda, MD). Heparinase was provided by Dr. Alfred Linker (VA Hospital, Salt Lake City, UT). Reference glycosaminoglycans were obtained from Dr. M. B. Mathews and J. A. Cifonelli (Dept. of Pediatrics, University of Chicago, Chicago, IL). Reagents were purchased from the following suppliers: steroids, prostaglandins, cholera toxin, dibutyryl cyclic AMP, cyclic AMP, dibutyryl cyclic GMP, cyclic GMP, cyclo eximide and concanavalin A from Sigma Chemical Co., St. Louis, MO; collagenase, form III, from Advance Biofactors, Lynbrook, Long Island, NY; Ionomycin from Dr. Edward Meyers, Squibb Institute for Medical Research, Princeton, NJ; A23187 from Calbiochem-Behring Corp., La Jolla, CL; cytochalasins from Aldrich, Milwaukee, WI; colchicine and vinblastine from Eli Lilly, Indianapolis, IN; gangliosides and ceramides from Supelco, Bellefonte, PA; isoproterenol hydrochloride from Winthrop Laboratories, New York, NY; and propranolol hydrochloride from Ayerst Lab, South Plainfield, NJ.

RESULTS

Thermal Activation of the Ligand

If aliquots of the cold ^{125}I-LRSC stock solution are diluted into binding buffer and if binding studies are performed directly with that solution at 26°C, <0.5% of the input radioactivity will bind to fibroblasts. By contrast, if the ligand solution is preincubated at 37°C for 30 min, cooled to 26°C and then presented to the cells, ~10% of the input radioactivity is specifically bound. Fig. 1 shows the effect on ligand binding of 30-min preincubations at different temperatures. It is evident that the ligand must be warmed above 35°C to promote significant binding and that binding is maximized when the ligand is heated to 42°C. Above 37°C, lathyritic rat skin collagen in dilute solution will begin to melt (5), i.e., chain separation will occur and the native triple helical molecule will be converted to random coil gelatin. Because the aim of the study was to examine the binding of the native collagen molecule, 37°C was chosen as the standard temperature for thermal activation of the ligand. The experiment of Fig. 2 shows that the binding capacity of the ligand increases rapidly during the initial 10 min of incubation at 37°C and that it approaches a constant value after 30 min. Thermal activation is not reversed by subsequently keeping the ligand at 4°C for several hours because ligand so treated does not require reheating to 37°C to bind efficiently to cells. Thus, routinely for binding studies, the ^{125}I-LRSC was diluted with binding buffer to a concentration of 10–20 ng/ml, incubated for 30 min at 37°C, cooled to 26°C, and cell binding studies were performed at the latter temperature.

Triple-helical collagen resists proteolytic digestion. To assess the native helicity of collagen activated at 37°C, I compared its resistance to proteolysis to that of native, unwarmed control substrates. Three different digestion protocols were used (see Materials and Methods); in each case the activated and control substrates were equally resistant to proteolysis (data not shown). Thermal activation of the ligand could thus be ascribed to two processes: (*a*) conformational changes within a molecule that remains triple helical; and (*b*) assembly of ligand molecules into larger aggregates. Process *a* is probably independent of ligand concentration, whereas the rate of process *b* is known to be directly proportional to the concentration of collagen (6–8). To determine which of these processes is responsible for thermal activation, I warmed ^{125}I-LRSC solutions at 14.2 μg/ml and at 12.7 ng/ml, respectively, at 37°C for 30 min. The more concentrated solution was then diluted to 12.7 ng/ml. Equal amounts of the respective ligand preparations were then added to replicate fibroblast cultures and specific binding with time was measured. Fig. 3 shows that both ligands bound to the fibroblasts but that the ligand activated at the 1,000-fold lower concentration bound more efficiently. This result suggests that conformational changes in the molecule are probably the basis for ligand activation and that polymerization of ligand beyond some limiting size results in less efficient binding.

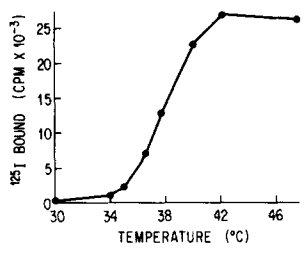

FIGURE 1 Effect of temperature on binding capacity of ligand. Stock ^{125}I-LRSC was diluted with binding buffer to give 6.3 × 10^4 cpm/ml and heated at different temperatures for 30 min. After cooling to 26°C, 2 ml of ligand solutions were added to each culture plate of 3T3 fibroblasts (2 × 10^6 cells). Specific binding was measured after 2 h at 26°C.

Effect of Ligand Concentration on Binding

Collagen binding to fibroblasts is a saturable process (2) but addition of collagen in excess of saturating amounts apparently decreases the total amount of specifically bound collagen. This is shown by the following experiment. Cultures received a fixed amount of ^{125}I-LRSC and increasing amounts of unlabeled LRSC. The cultures were incubated at 26°C for 2 h to achieve binding equilibrium, the amount of ^{125}I-LRSC bound was measured, and the total amount of labeled and unlabeled ligand specifically bound to the cells was calculated. Fig. 4 shows that saturation was approached after addition of 70 pm (20 μg) of unlabeled collagen but that addition of 140 pm of collagen caused the total amount of bound collagen to decrease by 20%. In hormone-receptor systems, such enhanced dissociation at high ligand concentrations is well known, and the phenomenon has been variously ascribed to experimental artifacts or to biologically relevant cooperative interations between receptor sites (see reference 9 for general discussion). Ligand-ligand aggregation is one experimental cause for the phenomenon and is a likely possibility in the collagen system,

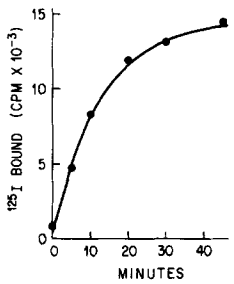

FIGURE 2 Time dependency of thermal activation of ligand. ^{125}I-LRSC was diluted with binding buffer to 6.1 × 10^4 cpm/ml and incubated at 37°C for the indicated times. The respective solutions of ligand were then cooled to 26°C, 2 ml of each solution was added to replicate 3T3 cultures (3 × 10^6 cells), and specific binding was measured after 2-h incubations at 26°C.

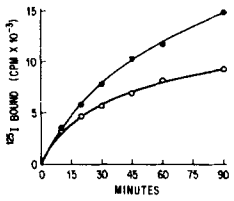

FIGURE 3 Effect of ligand concentration on thermal activation. ^{125}I-LRSC stock (14.2 μg/ml) was heated at 37°C for 30 min and then diluted into binding buffer to 12.7 ng/ml (○). ^{125}I-LRSC stock was diluted to 12.7 ng/ml with binding buffer and then heated at 37°C for 30 min (●). Replicate cultures received 2 ml (1.2 × 10^5 cpm) of the respective solutions of ligand, and specific binding at 26°C was measured at intervals.

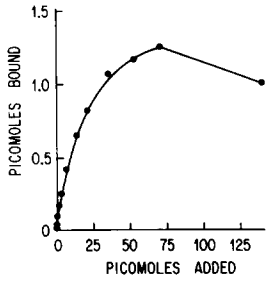

FIGURE 4 Binding as a function of ligand concentration. Increasing amounts of unlabeled LRSC (0–100 μg) were premixed with a fixed amount of ^{125}I-LRSC (1.38×10^5 cpm) in 2 ml of binding buffer. After activation at 37°C for 30 min, the solutions were added to replicate cultures and binding was measured after 2 h at 26°C. The picomoles of collagen added (radiolabeled and unlabeled) and specifically bound (radiolabeled and unlabeled) were then computed.

given that molecule's propensity for polymerization. In the experiment of Fig. 4, it is likely that some degree of ligand aggregation occurred at the highest concentration of added unlabeled collagen (20 μg/ml), both during the 30-min activation step at 37°C and during the 2-h incubation with cells at 26°C.

Negative cooperativity is generally ascribed to allosteric interactions between mobile receptors, and thus such interactions should be diminished or abolished in cells treated with cross-linking aldehydic fixatives. To the degree that an enhanced off-rate by added excess ligand depends upon negative cooperativity, the enhanced off-rate should be diminished in fixed cells. 3T3 fibroblasts fixed for 15 min at 26°C in 2% buffered paraformaldehyde will specifically bind ^{125}I-LRSC but binding is reduced by 80% and the off-rate in the absence of added LRSC is somewhat increased as compared to unfixed cells. However, the addition of excess (100 μg) unlabeled LRSC to labeled fixed cells enhances the off-rate to the same relative degree as for labeled unfixed cells (data not shown). Thus, the evidence does not favor negative cooperativity as an important mechanism for enhanced dissociation in the collagen binding system.

Test for Pinocytosis of Ligand

About 80% of the ^{125}I-LRSC bound to fibroblasts is removed when the cells are incubated with 270 U of bacterial collagenase for 15 min at 37°C. Cell layer morphology is unaltered by this treatment. Reasoning that pinocytosis of bound ligand should decrease the amount of radioactivity released by collagenase, I performed the following experiment. Fibroblasts were incubated with ^{125}I-LRSC for 60 min at 26°C, unbound ligand was removed by washing, and the cultures were then incubated in binding buffer at 37°C for 2 h. At intervals, replicate plates received collagenase as described above, and the amount of bound radioactivity remaining in these and control plates was measured. The data are presented in Fig. 5. The off-rate of the ^{125}I-LRSC in the control plates is greater than the off-rate recorded for cultures maintained at 26°C (2). This means that if the shift from 26° to 37°C after binding stimulated pinocytosis of ^{125}I-LRSC, the effect was outweighed by a relatively greater increase in the dissociation rate of the ligand. Moreover, the data of Fig. 5 show that throughout the 2-h "chase" at 37°C the fraction of bound ligand released by collagenase remained constant (~80%). Thus, with time, the bound ligand did not become less accessible to added collagenase and, by this criterion, significant pinocytosis of the ligand did not occur. Moreover, that fraction of radioactive ligand that was not released by the short incubation with collagenase could also have been bound to the cell surface.

Binding and Metabolism of the Fibroblast

The plasma membrane component which binds to the collagen has not been identified. By the following tests, the ligand does not appear to bind to collagens, glycosaminoglycans, or gangliosides on the surface of the fibroblast. (*a*) The rate and amount of ligand binding are unchanged when the cells are pretreated with bacterial collagenase, chondroitinase ABC or testicular hyaluronidase (2). (*b*) Pretreatment of 2×10^6 3T3 fibroblasts with 100 μg/ml of crude heparinase for 1 h at 37°C does not alter the binding reaction. (*c*) Addition to the cultures of 40 μg of hyaluronate, chondroitin 4-sulfate, chondroitin 6-sulfate, dermatan sulfate, type II keratan sulfate, heparin, heparan sulfate, ceramides, or gangliosides (G_{T1}; G_{D1a}; or G_{M1}), respectively, does not alter binding.

A variety of agents that modify fibroblast metabolism were tested for possible effects on the binding reaction (Table I). The agents were applied under conditions which allowed expression of their metabolic actions but which did not alter cell layer morphology, as judged by phase microscopy. None of the listed agents altered the binding of ^{125}I-LRSC (data not shown) when compared to control cells.

Although the microtubule modifying agents, colchicine and vinblastine, were without effect, the microfilament modifiers, cytochalasins B and D, caused inhibition of ^{125}I-LRSC binding.

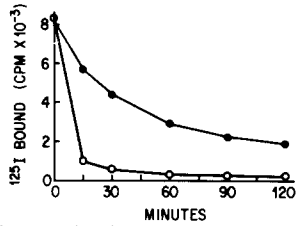

FIGURE 5 Accessibility of bound ligand to added collagenase. Replicate cultures were labeled with ^{125}I-LRSC (1.3×10^5 cpm) for 60 min at 26°C, washed, and then incubated with 2 ml of binding buffer at 37°C. At invervals, one set of cultures received 270 U of bacterial collagenase for 15 min. Radioactivity bound to cell layers in control cultures, (●) and collagenase-treated cultures (○) was then measured.

TABLE I

Agents Added to Fibroblast Cultures

	Concentration	Preincubation
		Hours at 37°C
Hydrocortisone	2×10^{-5} M	18
Cortisone	10^{-5} M	18
Prednisolone	10^{-5} M	18
Dexamethasone	2×10^{-5} M	18
Testosterone	10^{-5} M	18
β-estradiol	10^{-5} M	18
Arachidonic acid	1 μg/ml	1
Prostaglandins (E_1, E_2, F_{2a})	1 μg/ml	1
Cholera toxin	0.5 μg/ml	18
Dibutyryl cyclic AMP	1 mM	18
Cyclic AMP	1 mM	18
Dibutyryl cyclic GMP	0.25 mM	18
Cyclic GMP	1 mM	18
Isoproterenol	2.5 μg/ml	18
Propranolol	0.5 μg/ml	18
Ionophores:		
ionomycin	5 μM	1
A23187	15 μM	1
Colchicine	10^{-7} M	2
Vinblastine sulfate	5×10^{-8} M	2
Cycloheximide	25 μg/ml	18

After preincubations, standard 2-h binding assays were performed with the drugs present at the indicated concentrations.

The fibroblasts were preincubated with the cytochalasins for 90 min at 37°C, and the drugs were also present during the subsequent 2-h incubation with ^{125}I-LRSC at 25°C. At cytochalasin concentrations above 2.5 μg/ml, significant cell detachment occurred. At lower concentrations, some cell retraction and rounding without detachment occurred. At cytochalasin concentrations below 1 μg/ml the morphologic changes were minimal. Table II shows a comparison between the effects of the lower concentrations of the two cytochalasins on the morphology of the cell layers and the binding reaction. It is evident that cytochalasin D is the more potent drug with respect to both altering cell morphology and inhibiting binding.

The lectin concanavalin A interacts with carbohydrate moieties of membrane macromolecules, causing changes in the surface properties of cells (reference 10, for general review). 3T3 fibroblasts were incubated with different concentrations of concanavalin A, and a dose-dependent inhibition of ^{125}I-LRSC binding was demonstrated (Fig. 6). To determine whether the addition of concanavalin A reduced the number or affinity of the binding sites, I pretreated the cells with 8 μg/ml of the lectin and then incubated the cells in its presence with ^{125}I-LRSC and various concentrations of unlabeled LRSC, and then I analyzed ligand binding by a Scatchard plot. Fig. 7 shows that control and concanavalin-treated cells gave lines with similar slopes but that less ligand was bound at saturation in the concanavalin-treated system. This result indicates that concanavalin caused a reduction in the number of binding sites without altering their affinity. A similar result was obtained when a Scatchard plot of cytochalasin D inhibition of binding at 0.5 μg/ml was performed (data not shown).

TABLE II

Effects of Microfilament Modifiers

	Concentration	Cell retraction and rounding	Inhibition of binding
	μg/ml		%
Cytochalasin B	2.5	++	22.8
	1.0	+−	0
Cytochalasin D	1.0	++	68.4
	0.5	+−	50.0
	0.25	+−	29.3

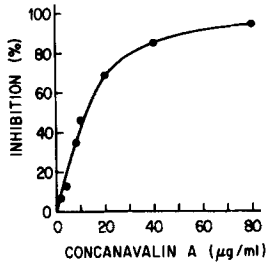

FIGURE 6 Inhibition of binding by concanavalin A. Replicate cultures were incubated with increasing concentrations of concanavalin A in binding buffer for 60 min at 37°C. Washed cultures then received activated ^{125}I-LRSC (1.3 × 10^5 cpm) and corresponding concentrations of concanavalin A in binding buffer. The cultures were incubated for 2 h at 26°C and specific binding was measured. Data are plotted as percent inhibition of binding measured in replicate control plates that did not receive concanavalin A.

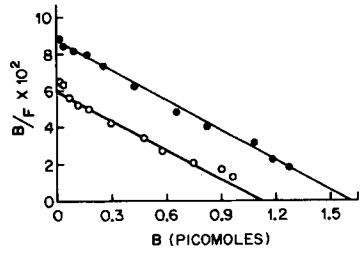

FIGURE 7 Scatchard plot of binding data in presence and absence of concanavalin A. Increasing amounts of unlabeled LRSC were premixed with fixed amounts of ^{125}I-LRSC and presented to cells (same conditions as for experiment of Fig. 4). One set of plates (○) received 8 μg/ml of concanavalin A 1 h before and during the 2-h labeling interval. Control cultures (●).

DISCUSSION

The ^{125}I-LRSC activated for binding by preincubation at 37°C for 30 min resists limited digestion with proteases. By this criterion the activated ligand retains triple helical structure. The warming requirement is not a consequence of radioiodination of the molecule because unlabeled LRSC also requires thermal activation to compete effectively for binding sites in inhibition assays (11). I judge that warming of the ligand at the physiologic temperature induces subtle conformational changes that do not include misalignment or separation of the three helical α chains. Activation of the ligand was routinely performed in highly dilute solutions (10–20 ng/ml). Ligand activated at a 1,000-fold greater concentration bound somewhat less well to fibroblasts. Warming at the higher concentration may have promoted polymerization of the ligand, and the latter process could have reversed conformational changes in individual molecules or produced molecular aggregates with decreased affinities for binding sites. Such ligand-ligand interactions rather than negative cooperativity are thought to be the basis for the decrease in total binding when a large excess of unlabeled collagen is added to the cultures as in Fig. 4. In Scatchard analyses, I have disregarded such anomalous data points and have derived the binding constants from a single straight line fit. From such plots the K_A approximates 10^8/M, and about 5 × 10^5 binding sites per cell are calculated. For comparison, insulin binds to the fibroblast at 24°C with a K_A of 10^9/M, but only 4 × 10^3 molecules of hormone bind per cell at saturation (12). Even with the large number of binding sites for collagen, I calculate that at saturation, no more than 8% of the surface area of the 3T3 fibroblast is covered by collagen.[1]

Collagen bound to the fibroblast surface dissociates at a very slow rate. The half-life of the bound complex at 26°C is about 2.5 h and, at 37°C, 46 min (2). Although remaining upon the cell surface for relatively long periods, the ligand is not actively pinocytosed, as judged by the test of undiminished accessibility to added collagenase.

The evidence does not favor the view that ^{125}I-LRSC binds to collagens, hyaluronate, or proteoglycans coating the fibroblast surface. Nor is the binding blocked by added gangliosides or ceramides. The soluble native ligand does not appear to bind to fibronectin on the cell surface (2, 11). The presumption is that the ligand binds to a still unidentified glycoprotein component of the plasma membrane.

A possible function for collagen binding could be the initiation of biochemical reactions which ultimately help regulate

[1] Computed to give a minimum for the surface area of the fibroblast and a maximum for the area covered by collagen. Measurements were made of 3T3 cells at confluence. The cell shape was assumed to be rectangular; irregular or long cytoplasmic projections were ignored and the two dimensions were taken from the main cell area. The average cell area calculated was 2.7 × 10^{-5}cm^2. Collagen was assumed to bind only to the fibroblast surface away from the plastic substrate. Only one collagen molecule was assumed to bind by its entire lateral surface to each cell binding site. The collagen surface in contact with the cell was considered to be a rectangle with the dimensions 1.5 × 300 nm. At saturation, collagen would thus cover an area of 2.25 × 10^{-6}cm^2, or approximately 8 per cent of the computed surface area.

collagen synthesis and secretion. In the hope of detecting such functional linkages, the fibroblasts were treated with several agents (Table I) which modulate collagen synthesis, turnover, and secretion (13–18), but none of the drugs altered the amount of bound ligand. The finding that binding was not diminished even after 18-h exposure to cycloheximide indicates that the cell binding site is not subject to significant turnover in growth-inhibited 3T3 fibroblasts.

The observation that collagen binding is inhibited by cytochalasins and concanavalin A can be interpreted in terms of the known effects of these agents on classical surface receptors. For example, Van Obberghen et al. reported that cytochalasins A and B decreased the binding of insulin and human growth hormone to human lymphocytes and that such binding was unaffected by colchicine and vinblastine. As in the case for collagen binding, the cytochalasins reduced the number of hormone binding sites without altering the binding affinities (19).

Concanavalin A has been shown to inhibit the patching and capping of immunoglobulin and concanavalin A receptors on lymphocytes (20, 21), and the effects of the lectin are partially reversed with colchicine and vinblastine (22). Thus, evidence has accumulated that the structure, mobility, and availability of surface receptors are dependent upon interactions with cytoskeletal elements (23, 24). This property of surface receptors is evidently shared by the collagen binding site.

The above-described characteristics of the collagen binding reaction are consistent with its participation in some phase of the extracellular maturation of collagen. A circumstantial case has been made for the binding reaction as an initiating and controlling event in the assembly of collagen molecules into fibrils (2). The central idea of the proposal is that the binding of procollagen or collagen molecules to a plasma membrane component segregates the collagenous molecules and helps to determine the spatial specificity of their assembly. Ligand-ligand interactions are thought to play a role in the detachment of the molecular aggregates from the cell surface, and the local concentration of soluble detached polymers could modulate, in turn, the number, size and off-rates of surface-bound units.

Although helical filaments will form "spontaneously" from pure solutions of collagen, actin, tubulin, keratin, and chromatin, the bonding properties of the respective subunits do not uniquely specify the geometry of packing in the filaments (25). The argument is made, therefore, that the specificity of packing of biological filaments in vivo is critically controlled by interactions with other cell-associated molecules (26). In that sense, microtubule organizing centers are identified as nucleation sites for tubulin assembly in the cytoplasm (27), and I propose that the collagen binding site fulfills an analogous role for collagen fibril assembly at the cell surface. Collagen types I, II, and III characteristically form periodic fibrils in vivo. The periodicity is due to the spatial order of packing in the fibril. Only native collagen types II and III inhibit binding to type I sites (11), providing circumstantial evidence that this binding reaction helps to determine the mode of molecular packing in periodic collagen fibrils. A precedent for cell-surface directed growth of biological filaments is provided by studies of cellulose biogenesis. In this case, the glucan polymers with a characteristic parallel-chain crystalline lattice elongate from complexes in the plasma membrane of higher plants and algae (28, 29), or they are extruded from the lipopolysaccharide wall of bacteria (30).

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