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Properties of Rat Erythrocyte Membrane Cytoskeletal Structures Produced by Digitonin Extraction: Digitonin-Insoluble β-Adrenergic Receptor, Adenylate Cyclase, and Cholera Toxin Substrate

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Summary. Rat erythrocyte plasma membranes have been extracted exhaustively with digitonin at low temperature, and the residual, detergent-extracted membrane cytoskeletal material is compared to that prepared with Triton X-100 with respect to protein, glycoprotein, phospholipid, and cholesterol content. Digitonin, a weaker detergent than Triton X-100, solubilizes only 26% of the phospholipids and none of the cholesterol. SDS-polyacrylamide gel electrophoresis reveals that differences between the proteins extracted by the two detergents are primarily quantitative. In terms of functional preservation, digitonin retains in the cytoskeleton 28% of the β-adrenergic receptor binding activity (with the balance accounted for in the supernatant), >90% of the adenylate cyclase and >90% of the 45,000 mol wt polypeptide cholera toxin substrate. The cytoskeletal-associated β-adrenergic receptor retains binding properties for antagonist and agonist which are identical to those of the native membrane receptor. The digitonin-extracted cytoskeleton containing the β-adrenergic receptor may provide a useful vehicle for the reconstitution of a hormone-sensitive adenylate cyclase.

Key words cytoskeleton · β-adrenergic receptor · adenylate cyclase · cholera toxin · digitonin · erythrocyte

Introduction

The hormone-sensitive adenylate cyclase system has been shown to consist nominally of three components, the catalytic adenylate cyclase, a guanylnucleotide-sensitive regulatory protein (*N*-protein), and a receptor moiety conferring specificity of the system to particular hormones. Interactions among these components have been determined by a series of genetic complementations and the use of several reconstitution systems [13, 17, 27, 33]. Recent work in this laboratory has involved the construction of a reconstitution system to investigate the interaction of the three components of the hormone-sensitive adenylate cyclase system with other protein and lipid components of the rat erythrocyte plasma membrane [20, 28–30]. Retention of the *N*-protein and the catalytic adenylate cyclase in cytoskeletal matrices generated with the nonionic detergent, Triton X-100, along with extraction and rebinding experiments under activating conditions, characterized distinct proteinaceous binding sites for these proteins. The present communication describes a cytoskeletal system prepared with digitonin that demonstrates association of adenylate cyclase, *N*-protein, and a sizeable population of the β-adrenergic receptor with cytoskeletal elements. The preparation is characterized with respect to its protein, glycoprotein, and lipid composition.

Materials and Methods

Digitonin was obtained from either Sigma or Fisher. ATP, trisodium phosphoenolpyruvate, aminophylline, D- and L-isoproterenol bitartrate, hydroxyethyl-piperazine sulfonic acid (HEPES), and phenylmethylsulfonylfluoride (PMSF) were purchased from Sigma. Guanylyl-5′-imidodiphosphate (Gpp(NH)p) was obtained from Boehringer-Mannheim. ^{125}I-Hydroxybenzylpindolol, ^{3}H-L-dihydroalprenolol, (2,8-^{3}H) cAMP, and (α-^{32}P) ATP were supplied by NEN. (α-^{32}P) NAD^+ was synthesized by published methods [9]. Adenylate cyclase assays were performed as previously described [29]. The 45,000 (45K) mol wt polypeptide of the putative guanylnucleotide regulatory protein (*N*-protein) was labeled with cholera toxin and (α-^{32}P) NAD^+ (5–7 Ci/mmol) according to [9]. Total phospholipid phosphorus was determined by the Bartlett procedure [3]. The phospholipids were separated on silica H plates (Applied Science) without binder, using $CHCl_3$:CH_3OH:CH_3COOH:H_2O (75:40:12:2) (vol/vol/vol/vol) as the mobile phase. Bands visualized with I_2 vapor comigrating with authentic standards were scraped and their phosphorus content was determined by the Bartlett procedure. Cholesterol determinations were made on the total lipid extract according to [36]. Inclusion of a digitonin precipitation step in this determination eliminated interference from Triton X-100 which might carry over through the organic solvent extraction. Protein determinations were made by the procedure of [37] to remove detergent interference. SDS-polyacrylamide gel electrophoresis and quantitative autoradiography were performed as described [18]. A Zeinah® laser densitometer was used to quantitate Coomassie Blue R-250 staining in polyacrylamide gels.

Rat Erythrocyte Membrane Preparation

Rat erythrocytes were obtained by cardiac puncture from young male Sprague-Dawley rats (75 to 100 g) into heparinized syringes.

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The blood was centrifuged at $1,500 \times g_{max}$ for 10 min at 4 °C and the supernatant and buffy coat removed. Two washes in 0.85% NaCl at 4 °C were followed by aspiration of the supernatant and the top layer of erythrocytes to assure removal of remaining leukocytes. Up to 20% of the red cells were sacrificed to exclude leukocytes with their associated proteases. Ghosts were prepared by lysis of the washed cells in at least ten cell volumes of 7.5 mM sodium phosphate buffer, pH 7.5, at 0 °C in the presence of 40 μg per ml PMSF. The supernatant was removed by suction, and the process was repeated two more times, with care being taken to remove any viscous pellet (aggregated white cells) before recentrifugation. The membranes were washed once in 5 volumes of ice-cold 20 mM HEPES-130 mM NaCl-5 mM KCl-5 mM $MgCl_2$, pH 7.4 (buffer A). One cell volume of buffer A was added to give a concentrated membrane preparation (4 mg/ml membrane protein).

Preparation of Digitonin-Cytoskeletons

Concentrated erythrocyte membrane preparations were extracted with 5 to 10 volumes of ice-cold buffer A containing 40 μg per ml PMSF and the desired concentration of digitonin. Stock solutions (5% wt/vol) of digitonin in buffer A were made by heating to boiling followed by clarification through Millipore GS (0.22 μm) filters and were stored at room temperature. A time course of extraction of the membranes indicated that no further changes took place after 15 min on ice. Membrane suspensions were centrifuged at $40,000 \times g_{max}$ for 15 min at 4 °C, and the supernatants were carefully removed with a Pasteur pipette. It proved unnecessary to centrifuge the preparation at higher *G*-forces. Pellets were resuspended by vortexing with the aid of a glass rod or by mild Dounce homogenization.

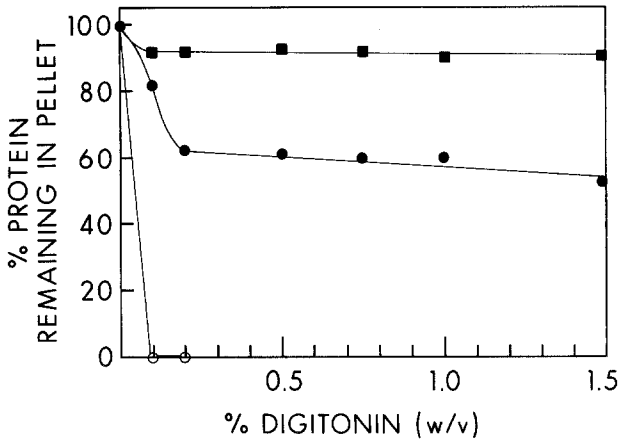

Fig. 1. Extraction of rat erythrocyte ghost membrane proteins with digitonin. Rat erythrocyte ghosts (4 mg/ml protein) were extracted with five volumes of Buffer A containing the indicated concentrations of digitonin for 20 min at 0 °C. The insoluble residue was collected at $40,000 \times g_{max}$ for 10 min. Aliquots corresponding to the same initial amount of membrane protein were electrophoresed on duplicate SDS-7.5% polyacrylamide slab gels. One gel was stained with Coomassie Blue R-250 while the other was processed to detect sialic acid [15]. Protein was quantitated by scanning the Coomassie Blue-stained gel at 550 nm band by inspection of the periodic acid-Schiff (PAS) reagent stained gel, (■) spectrin, actin; (●) Band 3; (○) PAS-staining bands

Results

Cytoskeletal structures (cytoskeletons) prepared by digitonin extraction of erythrocyte ghosts produced a very dense precipitate. When subjected to centrifugation over a 20–60% (wt/vol) sucrose gradient in buffer A layered over a 68% (wt/vol) sucrose shelf, the cytoskeletons collected at the shelf interface, well separated from unextracted membranes [32]. This was not surprising considering the amount of digitonin bound to the cytoskeletons as a complex with cholesterol (*see* Lipid Analysis). The digitonin cytoskeletons were, however, readily suspended and did not aggregate once dispersed although they would gradually settle spontaneously over longer time periods.

Protein Composition

Figure 1 shows a digitonin concentration curve of extraction of the major rat erythrocyte membrane proteins. The ionic and temperature conditions for the extractions were chosen to stabilize membrane cytoskeletal elements. Compared to Triton X-100, digitonin is a mild detergent. Only 45% of the total membrane protein was extracted with digitonin, while Triton X-100 removed 64% at maximal concentrations of detergent. The major Coomassie Blue-staining membrane proteins (Fig. 2) extracted differently at the maximal digitonin concentration. Spectrin and several other high mol wt proteins as well as actin remain firmly associated with the membrane. Band 3, and another protein of intermediate mol wt roughly corresponding to human band 4.1, are partially extracted. The two major glycoproteins which stain with periodic acid-Schiff reagent (for sialic acid) are removed completely with 0.2% or higher concentrations of digitonin and Triton X-100.

Comparison of the extraction of erythrocyte membranes with digitonin and Triton at 0 °C (Fig. 2) indicates that the two detergents tend to extract the same proteins but that Triton X-100 is more efficacious in their removal. Further extraction of digitonin pre-extracted cytoskeletons with Triton X-100 yields a residue identical to that of Triton X-100-extracted ghosts. Digitonin extraction of Triton X-100 pre-extracted cytoskeletons fails to remove any more protein.

Lipid Analysis

Table 1 compares the lipid content of Triton X-100- and digitonin-extracted erythrocyte membranes. While digitonin precipitates all of the membrane cholesterol in the cytoskeleton, 54% of membrane cholesterol is removed by Triton X-100 treatment. Sixty-seven percent of the total lipid phosphorus is extract-

able by Triton X-100 while only 27% is solubilized by digitonin. The major difference in extractability among lipid classes lies in the relative retention of sphingomyelin in the digitonin-cytoskeletons while more than one-half is solubilized by Triton X-100. Sequential extraction of membranes with digitonin and Triton X-100 resulted in further lipid and cholesterol removal only when digitonin treatment was followed by Triton X-100 extraction. Triton X-100 solubilized about 50% of the digitonin-cholesterol complex along with many of the phospholipids.

Analysis of the Components of the Adenylate Cyclase System. Cytoskeletal β-adrenergic Receptor

Our prime interest in characterizing the digitonin-cytoskeletons of erythrocytes lies in the fact that the binding of β-adrenergic ligands occurs in the presence of this detergent. Twenty to thirty percent of the measured β-receptor binding activity remains associated with the cytoskeleton following extraction with digitonin, with the balance of the binding activity found in the supernatant. This occurs even after several extractions with maximal detergent concentrations, followed by several washes with detergent-free buffer. The guanylnucleotide binding protein content and the adenylate cyclase activity can also be characterized in the same preparation.

A digitonin concentration curve of extraction of the β-adrenergic receptor binding activity from rat erythrocyte ghosts is presented in Fig. 3. At maximal concentrations of digitonin one-third of the receptor remains associated with the digitonin-cytoskeleton. Similar results were obtained when the β-adrenergic receptor was prelabeled with ^{125}I-hydroxybenzylpindolol prior to solubilization. Receptor not detected in the cytoskeleton can be accounted for in the supernatant of the detergent extraction (Fig. 3). Pretreatment of the membranes with sotalol (antagonist) or

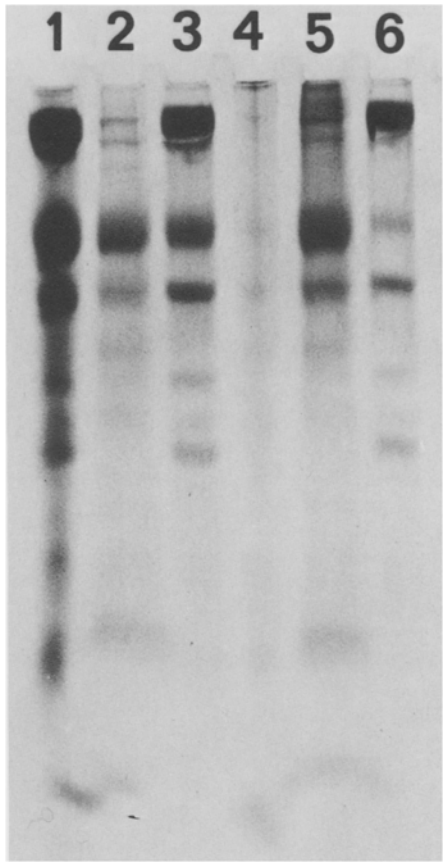

Fig. 2. Comparison of Triton X-100 and digitonin extraction of rat erythrocyte membrane proteins. Rat erythrocyte ghosts (4 mg/ml protein) were extracted with one volume of 2% of either digitonin or Triton X-100 in Buffer A at 0 °C for 20 min. Soluble material was separated by centrifugation at 150,000 × g_{max} for 20 min. Pellets were resuspended to the extraction volume. Aliquots corresponding to the same amount of starting membrane protein were electrophoresed on an SDS-7.5% polyacrylamide slab gel and stained with Coomassie Blue R-250. (*1*): Original membranes (150 μg); (*2*): digitonin supernatant (from 75 μg original membrane protein); (*3*): digitonin pellet (from 75 μg original membrane protein); (*4*): Triton X-100 supernatant as in *2*; (*5*): Triton X-100 supernatant precipitated with trichloroacetic acid before electrophoresis, otherwise as in *4*; (*6*): Triton X-100 pellet as in *3*

Table 1. Lipid and protein composition of cytoskeletons

Membrane component	Cell fraction		
	Membranes	2% Triton X-100 cytoskeletons	1% Digitonin cytoskeletons
Total lipid phosphorus[a] (%)	1750 (100)	583 (33)	1297 (74)
Sphingomyelin	227 (100)	99 (44)	220 (97)
Phosphatidylcholine	718 (100)	367 (51)	557 (78)
Phosphatidylserine + phosphatidylinositol	297 (100)	70 (24)	194 (65)
Phosphatidylethanolamine	508 (100)	47 (9)	324 (64)
Cholesterol[a] (%)	1077 (100)	498 (46)	1077(100)
Protein (%)	1 mg (100)	0.36 mg (36)	0.55 mg (55)

[a] nmol per mg original membrane protein. Percentages are expressed with respect to original membranes.

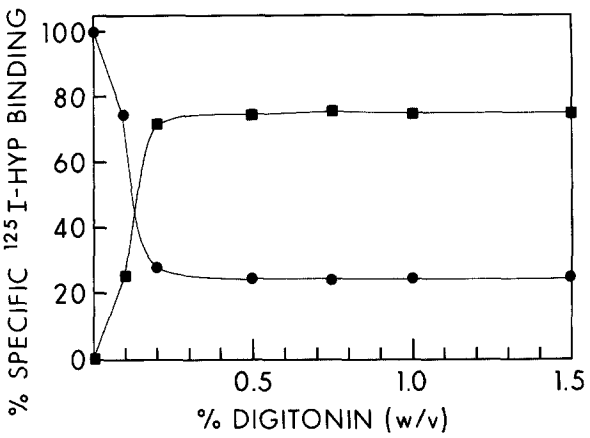

Fig. 3. Digitonin extraction and recovery of ^{125}I-hydroxybenzylpindolol (^{125}I-HYP) binding activity from rat erythrocyte membranes. Rat erythrocyte ghosts (4 mg/ml protein) were extracted with two volumes of 1.5% digitonin in Buffer A at 0° for 15 min. Soluble receptor was separated from the membranes by centrifugation at $150{,}000 \times g_{max}$ for 20 min. The pellet was resuspended in the original volume of Buffer A. β-adrenergic receptor binding activity was assayed by incubation with 20 pM ^{125}I-HYP $\pm 10^{-5}$ M DL-propanolol for 20 min at room temperature (0 °C for soluble receptor). Bound ligand was quantitated by filtration of the particulate receptor over GF/C Whatman glass fiber filters and gel filtration of the soluble receptor over G-25 Sephadex at 0 °C collecting the excluded column volume. Specific ^{125}I-HYP binding - (●) membrane pellet; (■) supernatant

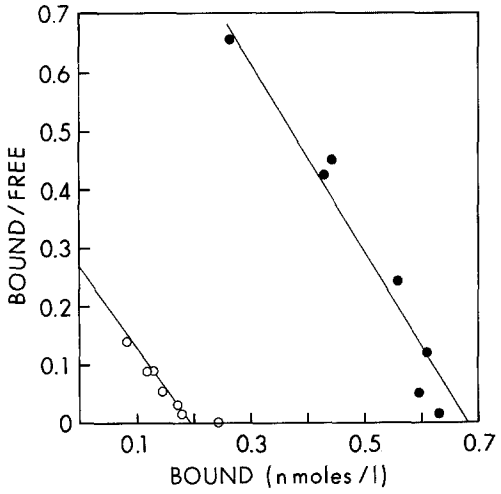

Fig. 4. Scatchard analysis of binding of ^{3}H-dihydroalprenolol to rat erythrocyte membranes and digitonin-extracted membranes. Rat erythrocyte ghosts (4 mg/ml protein) were extracted with digitonin as described in the legend to Fig. 1. The extracted membranes were washed once with ten volumes of detergent-free Buffer A and then resuspended in twice the original volume of Buffer A, using 50 µl in a 1-ml assay volume at room temperature. Specific binding was defined as that label displaced by 10^{-5} M DL-propanolol. Nonspecific binding for both filters and membranes was removed from the data before the Scatchard analysis. Control membranes were processed similarly in the absence of detergent. (●) Unextracted control membranes; (○) digitonin extracted membranes

L-isoproterenol (agonist) prior to extraction with digitonin did not result in alterations in the distribution of detergent-soluble and detergent-insoluble receptor. The substitution of ^{3}H-dihydroalprenolol for ^{125}I-hydroxybenzylpindolol gave a similar pattern for digitonin extraction of the β-adrenergic receptor, but with a lower nonspecific binding component. Scatchard analyses [31] of ^{3}H-L-dihydroalprenolol binding to the membrane and cytoskeletal receptors show parallel linear curves with a K_D of 1.5 nM for the tritiated ligand with the only effect of digitonin extraction being that of reducing the total number of binding sites to 28% of the number of membrane sites (Fig. 4). Displacement curves of the ^{3}H-dihydroalprenolol from the membrane-bound and cytoskeletal-associated receptor by L-isoproterenol or DL-propranolol are superimposable with IC_{50} values of 2.8 µM and 1.5 nM, respectively. Thus, the receptor found associated with digitonin cytoskeletons appears to possess most if not all of the binding properties of the membrane-bound form.

The association of the β-adrenergic receptor with the digitonin-cytoskeleton may be mediated either by attachment of the receptor to the proteinaceous cytoskeleton or by a nonspecific adsorption of the solubilized receptor to the residual lipid in the digitonin-cytoskeleton. The latter possibility was rendered unlikely by the lack of incorporation of labeled, soluble receptor into unlabeled digitonin-cytoskeletons (Table 2). Furthermore, labeled, soluble receptor incorporated into liposomes made from rat erythrocyte lipids could be completely solubilized by treatment with digitonin. Therefore, it appears likely that the association between the β-adrenergic receptor and the digitonin cytoskeleton is at least partially mediated by protein-protein interactions.

Table 2. Solubilized receptor does not adsorb to digitonin cytoskeletons

Preparation	cpm
Endogenous receptor in cytoskeleton	24,000
Soluble receptor added	317,668
Soluble receptor adsorbed to cytoskeleton	2,423
Soluble receptor adsorbed to cytoskeleton in the presence of 10^{-5} M DL-propanolol	2,834

Unlabeled digitonin-extracted cytoskeletons with the noted capacity of ^{125}I-HYP binding at the given input cpm of labeled receptor were incubated in Buffer A for 30 min at 0 °C with ^{125}I-HYP-labeled receptor which had previously been separated from free ligand on a Sephadex G-50 column at 4 °C. Cytoskeletal-associated receptor was estimated by filtration through GF/C filters and washing with ice-cold Buffer A. The values shown are corrected for filter binding of receptor. The addition of 10^{-5} M DL-propanolol was used to control for ligand dissociation and rebinding to residual cytoskeletal receptor.

Cytoskeletal Cholera Toxin Substrate

The 45,000 mol wt (45K) polypeptide cholera toxin substrate, a subunit of the putative guanylnucleotide regulatory component, has been shown by Triton X-100 extraction and direct binding studies to be functionally associated with the cytoskeleton in rat and human erythrocytes [28, 30]. The weaker detergent, digitonin, fails to remove an appreciable amount (less than 15%) of the 45K polypeptide from either rat reticulocytes or erythrocytes or pigeon erythrocyte membranes under the standard conditions described in this communication, which include the presence of serine protease inhibitors at 0 °C (Fig. 5). The cholera toxin-stimulated adenylate cyclase also remains associated with the digitonin-extracted cytoskeleton.

Cytoskeletal Adenylate Cyclase

While the basal activity of the rat erythrocyte adenylate cyclase is unstable to digitonin treatment, the Gpp(NH)p/isoproterenol-prestimulated adenylate cyclase activity remains associated with the cytoskeleton (Fig. 5). Enzyme activity was absent from the detergent supernatant. No adenylate cyclase activity could be detected in unstimulated cytoskeletons treated with either NaF or Gpp(NH)p/isoproterenol in the enzyme assay. It has been shown that adenylate cyclase does not bind to Triton X-100-extracted cytoskeletons unless the enzyme is first activated with NaF, Gpp(NH)p, or Gpp(NH)p or GTP/isoproterenol [29].

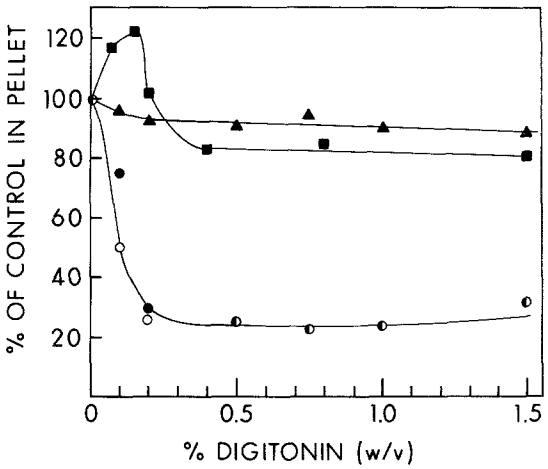

Fig. 5. Extraction of rat erythrocyte adenylate cyclase components with digitonin. Rat erythrocyte ghosts (4 mg/ml protein) were extracted with digitonin as described in the legend to Fig. 1. (■) Membranes pretreated at 30 °C for 10 min with 100 μM Gpp(NH)p and 10^{-5} M L-isoproterenol, washed, extracted with the indicated concentrations of digitonin, and assayed for adenylate cyclase activity. (▲) Membranes labeled with ^{32}P-NAD$^+$ and cholera toxin as in [18], extracted, and assayed for 45K polypeptide by SDS-polyacrylamide gel electrophoresis. (●) Membranes preincubated with ^{125}I-HYP, $\pm 10^{-5}$ M DL-propanolol, washed, extracted, and assayed by filtration for ^{125}I-HYP binding. (○) Membranes extracted, washed, and assayed for ^{125}I-HYP binding activity $\pm 10^{-5}$ M DL-propanolol

Discussion

Extraction of biological membranes with nonionic detergents has proven to be a useful tool in the study of the interactions of various membrane components. In particular, the study of the cytoskeleton and other specialized membrane-associated structures has been facilitated by this technique. Membrane residues obtained following extraction with Triton X-100 have been characterized and employed by various groups [4, 5, 7, 8, 10, 26, 38]. Digitonin, a milder nonionic detergent has been used for the separation of subcellular organelles [1, 24, 39], for disrupting the permeability barrier of cells [2, 16], and for solubilizing specific plasma membrane components such as the β-adrenergic receptor [22] and the neuroleptic receptor [35]. Using Triton X-100, we have previously provided evidence for a partial cytoskeletal association of the catalytic and regulatory components of the adenylate cyclase system [20, 30]. This detergent, however, inactivated most of the β-adrenergic receptor binding activity, and therefore did not permit study of the possible association of the receptor with the cytoskeleton. The use of digitonin reveals a greater degree of association of the catalytic and regulatory components of the adenylate cyclase system with the cytoskeleton. A significant (sub)population of the β-adrenergic receptor is also shown to be present in the same detergent residue, with the balance detectable in the detergent extract.

The differences between the solubilization patterns with digitonin and Triton X-100 of both lipid and protein may rise from the dissimilar ways in which the two detergents interact with membranes. While the Triton series of detergents seems to solubilize lipids and hydrophobic proteins in a manner primarily dependent upon the HLB (hydrophilic/lipophilic balance) number of the detergent, the steroidal glycoside, digitonin, has the property of forming stable stoichiometric complexes with cholesterol as well as micellar complexes with phospholipids. The ability of digitonin to disrupt a membranous structure is roughly proportional to the membrane cholesterol content, i.e., plasma membrane > lysosomes > outer mitochondrial membrane > inner mitochondrial membrane. These differences have been exploited in biochemical separations of subcellular structures [1, 14, 24, 39] and various membrane fractions [6, 23]. Hepatocytes made permeable by treatment with digitonin retain

the morphological characteristics of internal organelle structure as well as the cortical actin meshwork and intracellular intermediate filaments [6].

In the erythrocyte membrane system described in this communication, digitonin is selective in its action on the removal of protein and lipid. Three major groups of proteins could be distinguished on the basis of their extractability with digitonin: (i) digitonin-insoluble proteins such as spectrin, actin, ankyrin, the Gpp(NH)p-activated adenylate cyclase and the 45K mol wt cholera toxin substrate; (ii) proteins of which a subpopulation is soluble in digitonin such as band 3, and band 4.1, band 7 and the β-adrenergic receptor; and (iii) proteins completely solubilized by digitonin such as the sialic acid-containing glycoproteins of the red cell membrane.

The digitonin insolubility of certain erythrocyte membrane proteins can be correlated with their tendency to associate with certain other proteins rather than with lipid domains. The ankyrin-spectrin-actin complex has been shown to interact with a subpopulation of band-3 molecules to form a supramolecular network which dissociates only under specified conditions [25]. Digitonin fails to disrupt this meshwork as well as the interaction of the adenylate cyclase and the 45K mol wt cholera toxin substrate, which we have previously shown by reconstitution experiments to bind to proteinaceous components in the cytoskeleton [28]. Digitonin is capable, however, of extracting components from the membrane lipid matrix as in the solubilization of lysosomal membrane enzymes [24], outer mitochondrial membrane proteins [23], the noncytoskeletal population of band 3, the lipid-associated glycoproteins of the erythrocyte membrane and liposomal β-adrenergic receptor *(this communication)*. Thus, the nonextractability of a sizable portion of the β-adrenergic receptor and the observation that added exogenous receptor fails to bind to digitonin-extracted cytoskeletons suggests that at least a certain proportion (about one third) of the receptor is retained via protein-protein interactions other than by nonspecific association with lipids.

The digitonin-extracted cytoskeletons also contain a considerable amount of bound lipid. The presence of this lipid may help to account for the high activity of adenylate cyclase found in these preparations, whose activity has been shown to be influenced by certain lipids [19, 21]. A strict requirement for lipid in the binding of these components and the 45K mol wt cholera toxin substrate is unlikely since previous work in this laboratory has defined primarily proteinaceous binding sites.

The presence of a cytoskeleton is not obligatory for the function of the components of the adenylate cyclase system since hormonal activation can occur in a purely lipidic environment [12]. However, modulation of hormonal responses in cell membranes is markedly influenced by the state of assembly of this structure [20]. The decreased size and the dissociation from the cytoskeleton of the *N*-protein upon activation with guanylnucleotides or NaF has been proposed as modulatory step in the hormonal activation of adenylate cyclase [28]. A recent report using fluorescence polarization techniques [11] suggests that the β-adrenergic receptor in the antagonist occupied state is restricted in its mobility in the plane of the membrane by cytoskeletal elements. Agonist would presumably function to dissociate the receptor-cytoskeleton complex to facilitate interaction with the catalytic adenylate cyclase, and may thus be relevant to processes which regulate the number and affinity of receptors. Such a scheme would cast the cytoskeleton in the role of an inhibitory (restrictive) mechanism to control access of membrane components to one another.

The presence of digitonin-soluble and digitonin-insoluble fractions of the β-adrenergic receptor may reflect an equilibrium distribution of the receptor between an association with the cytoskeleton and the bilayer. Thus, different extractability of β-adrenergic receptor from various tissues [34] may imply a different degree of association with the cytoskeleton. The digitonin extraction technique may prove useful in determining the extent of cytoskeletal interactions of the β-adrenergic receptor under various physiological conditions in whole cells. This might allow biochemical access to the mechanism previously only inferred by the use of inhibitors which are not necessarily specific in their action. Finally, in addition to providing a tool with which to probe cytoskeletal interactions with a membrane receptor, the digitonin-extracted cytoskeleton containing β-adrenergic receptor binding activity may serve as a vehicle for recoupling the hormonal response of adenylate cyclase. Since association of the β-adrenergic receptor with the cytoskeleton does not appear to alter its binding affinity for several agonists and antagonists, an important goal for this system will be the recoupling of the hormonal process on the cytoskeletal matrix. The interaction of the other components of the adenylate cyclase system with the cytoskeleton have already been partially characterized [20, 28–30].

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