Noncovalently and Covalently Bound Lipid on the Receptor for Immunoglobulin E

Jean-Pierre Kinet,* Rodolfo Quarto, Ruy Perez-Montfort,‡ and Henry Metzger

Section on Chemical Immunology, Arthritis and Rheumatism Branch, National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, Maryland 20205

Received May 7, 1985

ABSTRACT: We describe experiments which indicate that lipids interact with the receptor for immunoglobulin E (IgE) in several ways. Evidence for loosely bound lipid comes from observations on the special conditions that are required in order to oxidatively iodinate the α chain of the receptor in IgE–receptor complexes. Evidence for tightly, but still noncovalently, bound lipids comes from studies on the lipids required to inhibit dissociation of the subunits of the receptor in micellar detergent. Finally, biosynthetic studies indicate that the subunits of the receptor contain ester-linked fatty acids. The latter modification appears to take place on receptors that have already been inserted into the plasma membrane.

There are many examples of membrane proteins whose activity is influenced by their interaction with the lipids in the bilayer (Sandermann, 1978). Although the plasma membrane receptor for immunoglobulin E (IgE)[1] cannot yet be assigned a specific biochemical function that can be monitored, we have made a variety of observations which indicate that this protein is associated with lipid moieties.

All of our studies have utilized rat basophilic leukemia cells (Eccleston et al., 1973) of the 2H3 line (Barsumian et al., 1981). The receptors for IgE on these cells (and on normal rat peritoneal mast cells) have been shown to consist of four polypeptide chains (Metzger et al., 1983): a 45-kDa α chain to which the IgE binds, a 33-kDa β chain, and two, disulfide-linked, 9-kDa γ chains. An unusual feature of this tetrameric complex is its instability in micellar detergent (Rivnay et al., 1982; Kinet et al., 1985). Several of the experiments described here were related to our investigations of this instability (Kinet et al., 1985).

Materials and Methods

Proteins and Cells. Monoclonal mouse anti-dinitrophenyl-IgE from hybridoma H1-DNP-e-26.82 (Liu et al., 1980) was prepared as described previously (Holowka & Metzger, 1982). Mouse IgE was iodinated with the chloramine-T method (McConahey & Dixon, 1966). Conjugation of the IgE with azobenzenearsonate groups has been described (Kanellopoulos et al., 1979). Cells from the rat basophilic leukemia cell line (2H3 line) were maintained as described (Barsumian et al., 1981).

Detergents and Lipids. The zwitterionic detergent 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS) was from Calbiochem-Behring Corp. (La Jolla, CA). *n*-Octyl β-D-glucopyranoside (octyl glucoside) was from Aldrich Chemical Co., Inc. (Milwaukee, WI). Octylphenoxypoly(ethoxyethanol) (Triton X-100) was obtained from Sigma Chemical Co. (St. Louis, MO).

Preparation and quantitation of the tumor-derived lipids and the lipids from rabbit liver have been described (Rivnay & Metzger, 1982). The other lipids used were from commercial sources: soybean lecithin (type IV S, Sigma), phosphatidylcholine (from bovine liver, no. 840055, Avanti Polar Lipids, Birmingham, AL), phosphatidylethanolamine (from bovine liver, no. 840026, Avanti), phosphatidylinositol (from bovine liver, no. 840042, Avanti), dipalmitoylglycerol (D2636, Sigma), and cholesterol (C8253, Sigma). To minimize oxidation, the solutions of lipids in chloroform were stored in sealed vials at −20 °C, freed of solvent by a stream of N_2, added to the detergent solution, and used the same day.

Extrinsic, Intrinsic Labeling. The procedures we used to iodinate the purified receptors with ^{125}I employing chloramine-T (McConahey & Dixon, 1966) were the same as those described (Alcaraz et al., 1984) unless noted otherwise. The procedure for biosynthetic labeling of cells with [^{35}S]-methionine has likewise been published (Kinet et al., 1983). For labeling with radioactive lipids, we somewhat varied the protocols throughout these studies but found the following most satisfactory: 1.5×10^7 cells grown in a 75 cm^2 stationary flask were washed and incubated in standard medium containing, however, only 0.1% fetal calf serum instead of the usual 20%. The radioactive fatty acid in dimethyl sulfoxide (final concentration 2% v/v) was added (1–5-mCi flask), and after 1 h, the concentration of serum was raised to 1%. The incubation was continued for 3 more h before the cells were harvested. For some experiments, IgE was added prior to the lipid; in most, afterward. The fatty acids used were [9,10-(N)-^{3}H]palmitic acid (50 Ci/mmol), [9,10(N)-^{3}H]myristic acid (52 Ci/mmol), and [9,10(N)-^{3}H]oleic acid (4.8 Ci/mmol), all from Amersham.

Immune precipitates of purified receptors were analyzed on polyacrylamide gels as before (Perez-Montfort et al., 1983). In those experiments in which we wished to extract all noncovalently bound lipids, the immune precipitates were extracted with 0.5 or 1 mL of chloroform/methanol (2:1) at room temperature for 1–2 h. The precipitates were centrifuged and washed at 4 °C with ether/ethanol (1:1). After centrifugation, the extraction and washing were repeated, and the precipitate was then analyzed. In some experiments, we analyzed the counts in the bands from the polyacrylamide gels, essentially as described by Schmidt et al. (1979). Slices of the gel were extracted with KOH/methanol; the extracts were acidified

‡Present address: Departmento de Inmunologia, Instituto de Investigaciones Biomedicas, Universidad Nacional Autonoma de Mexico, 04510 Mexico D.F.

[1] Abbreviations: IgE, immunoglobulin E; CHAPS, 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate; kDa, kilodalton(s); PS, phosphatidylserine; PE, phosphatidylethanolamine; PC, phosphatidylcholine; PI, phosphatidylinositol; SM, sphingomyelin; DG, dipalmitoylglycerol.

with HCl and extracted twice with 4 mL of chloroform. The chloroform solution was dried with N_2 and the residue dissolved in 100 μL of chloroform/methanol (1:1). The solutions were analyzed by thin-layer chromatography on Kodak 13179 silica gel sheets with chloroform/methanol/water (65:25:4) along with standards.

Solubilization and Purification of Receptor–IgE Complexes. Receptor–IgE complexes were solubilized such that the ratio of micellar detergent to micellar lipids (ρ) of the resulting extract was approximately 2 (Rivnay & Metzger, 1982). When anti-dinitrophenyl mouse IgE was used (most experiments), the complexes were purified on a (trinitrophenyl)lysyl-Sepharose column (Holowka & Metzger, 1982); when arsonylated rat IgE or mouse IgE was used, a rabbit anti-benzenearsonate column was employed (Kanellopoulos et al., 1979).

Analysis of Receptors. After incubation in the desired solvent, the receptor–IgE complexes were immunoprecipitated and electrophoresed in sodium dodecyl sulfate on polyacrylamide gels, and the radioactivity in the components was assessed by densitometry of autoradiographs (iodine) or by counting of gel slices (^3H, ^{35}S) exactly as described previously (Kinet et al., 1985). The molecular weights of the labeled components were estimated from a scale based on the standards electrophoresed simultaneously.

RESULTS

Loosely Bound Lipids. On intact cells, the α chain of the receptor cannot be surface-iodinated when the receptor is occupied with IgE (Conrad & Froese, 1976). Similarly, when IgE–α-chain complexes are isolated, the α chain cannot be efficiently labeled by oxidative iodination even when such iodination is performed in the presence of a noniodinatable (and therefore noncompeting) mild detergent (Pecoud & Conrad, 1981). These findings are unexpected because compositional analysis shows that there are some 12 tyrosines per α chain (Kanellopoulos et al., 1980.)

It now appears that the unavailability of the tyrosines is due to lipid loosely bound to the receptor when the latter is isolated by procedures that preserve its tetrameric structure. Cells bearing mouse anti-dinitrophenyl-IgE were solubilized with 10 mM CHAPS at 5×10^7 cells/mL. The extract was centrifuged, and aliquots of the supernatant were applied to several batches of (trinitrophenyl)-ε-aminolysyl-Sepharose. The columns were washed by using different protocols, and the IgE–receptor complexes from each were eluted with 10 mM (dinitrophenyl)-ε-aminocaproate. The eluates were then oxidatively iodinated, the unbound iodine was removed by centrifugation through Sephadex 25, and the effluent was reacted with anti-IgE. The immune precipitates were then analyzed on gels. Table I indicates typical results. As shown by experiment 1, in the presence of the solvent we originally described as useful for stabilizing the receptor (Rivnay et al., 1982), the α chain of the IgE–receptor complex fails to become iodinated. Figure 2 (lane 2) in Rivnay et al. (1982) and Figure 1 in Perez-Montfort et al. (1983) show gels of similar experiments. When the affinity columns are washed with micellar detergent alone (experiment 2), the β and γ chains of the receptor progressively dissociate (Rivnay et al., 1982; Perez-Montfort et al., 1983). Nevertheless, the α chain cannot be iodinated, showing that it is not simply the presence of the β and γ subunits which prevents iodination of α. This solvent is comparable to the one used by Pecoud & Conrad (1981) in which they also observed failure of the α chain in the solubilized IgE–α-chain complex to become iodinated, using lactoperoxidase.

Table I: Iodination of IgE–Receptor Complexes

expt	condn used for washing column-bound complexes: [CHAPS] (mM)	tumor lipids (mM)	time (h)	additions prior to iodination of eluted receptors	receptor components obsd[a]
1	10	2	48	none	α, *β, *γ
2	10		48	none	α
3	2		48	none	α, *β, *γ
4[b]	10		0.017		
	2		48	none	*α, *β, *γ
5[b]	10		0.017	CHAPS (10 mM) ± lipids (2 mM)	
	2		48		α, *β, *γ

[a] An asterisk prior to the component indicates substantial incorporation of radioactive iodine. The presence of uniodinated α chains can be determined in several ways: by the presence of β and γ in the anti-IgE immune precipitate, by an assay for soluble receptor–IgE complexes (Rossi et al., 1977), or by biosynthetic labeling of the cells (Holowka et al., 1980; and Figure 1). In all experiments, the bound IgE also became labeled. [b] The column-bound complexes had already been washed with 10 mM CHAPS/2 mM lipids.

In submicellar detergent alone (i.e., without lipids), the IgE–receptor complex is soluble and quite stable (Alcaraz et al., 1984; Kinet et al., 1985). Again, however, the α chain fails to incorporate iodine (experiment 3), demonstrating that the absence of detergent micelles is not per se sufficient for the α chain to become modified. However, when the column-bound receptors are briefly (e.g., 1 min) exposed to micellar detergents without lipids, and then maintained in submicellar detergent, excellent iodination of α (as well as of the other subunits) is observed (experiment 4).

Figure 1 presents the data from a more complete analysis of this phenomenon. Panel A compares the labeling of α when the IgE–receptor complex has been "stripped" for 1 min with varying amounts of lipid-free micellar detergent and is then maintained in submicellar detergent throughout washing and elution.

The maximal iodination achieved (30–35% of the total iodine incorporated into the subunits of the receptor, Figure 1A) is roughly what would be expected on the basis of the ratio of tyrosines in α (Kanellopoulos et al., 1980) to those in the sum of α, β, and γ (G. Alcaraz et al., unpublished results). That the increase in the ratio of labeled α to labeled $\alpha\beta\gamma_2$ is not simply due to dissociation of $\beta\gamma_2$ during the wash with micellar detergent is documented by the data in panels B and C. The closed squares (panel B) show that the ratio of counts in β and γ_2 relative to IgE remains constant, whereas the ratio of counts in α to those of IgE substantially increases (open squares). Furthermore, when an assay is used that does not depend on the ratios of counts in the extrinsically labeled chains, i.e., by intrinsic labeling, it can be seen (panel C) that there is no change in the ratio of β to α. [We have previously demonstrated that β and γ_2 always dissociate in unison (Kinet et al., 1985).] As shown in Table I (and the open circle in Figure 1A), if micellar detergent with or without phospholipids is added to a solution of stripped IgE–receptor complexes prior to the iodination, the α chain again fails to become labeled. These results as well as other data suggest that the reason the α chain cannot be efficiently iodinated in the native IgE–receptor complex is that it is associated with loosely bound lipid (Discussion).

Tightly Bound Lipid. In a previous study, we examined the influence of various experimental conditions on the dissociation of the subunits of the receptor by detergent (Kinet et al., 1985). Such a quantitative study became practical because we were

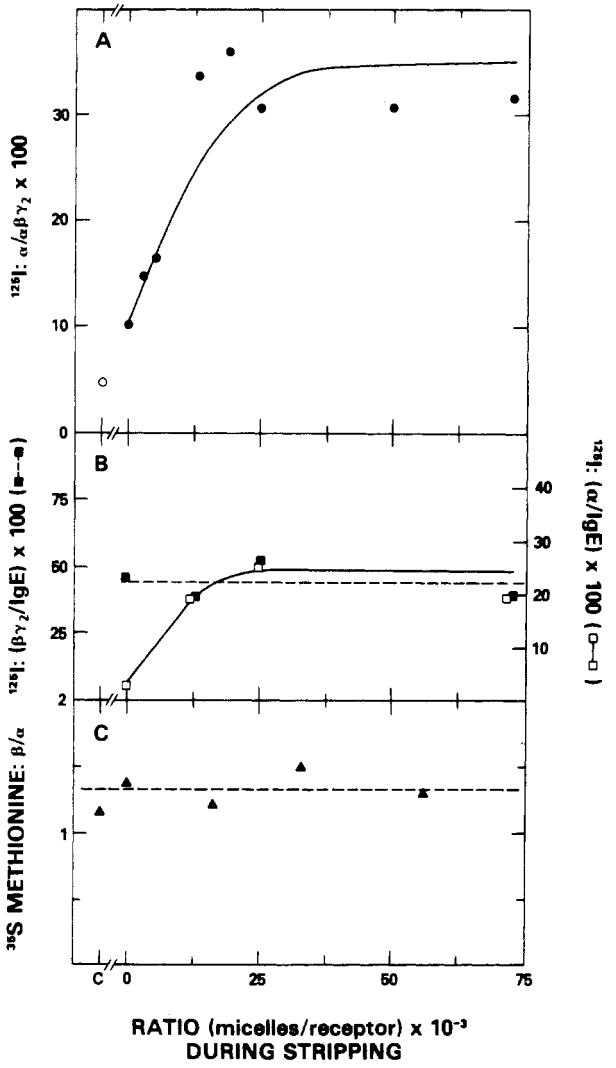

FIGURE 1: Incorporation of ^{125}I into the α chain of the receptor for IgE as a function of the exposure of the IgE–receptor complexes to detergent micelles. IgE–receptor complexes were extracted from unlabeled cells (panels A and B) and from cells biosynthetically labeled with [^{35}S]methionine (panel C). The extract was centrifuged and the supernatant applied to a (trinitrophenyl)-ϵ-aminolysyl-Sepharose column (50 μL to 600 μL of packed beads). The column was washed first with 200 bed volumes of 10 mM CHAPS/2 mM liver phospholipid then with a variable amount (0–10 mL) of 10 mM CHAPS at 4 °C for 1 min (stripping), and finally with 20 bed volumes of 2 mM CHAPS. The column was eluted with 10 mM (dinitrophenyl)-ϵ-aminocaproate in 2 mM CHAPS and the eluate oxidatively iodinated (panels A and B). After centrifugation through Sephadex G25, the IgE–receptor complexes were immunoprecipitated with anti-IgE and the precipitates analyzed on 12.5% polyacrylamide gels. Radioautographs of the dried gels were analyzed by densitometry, and the radioactivity associated with the different components was quantitated. (A) Incorporation of ^{125}I into the α chain relative to the receptor as a function of the total number of micelles passed through the column during the stripping procedure relative to the number of receptor molecules bound to the column. The open circle represents a control experiment in which the receptor has been eluted from the column in a lipid-containing buffer (10 mM CHAPS/2 mM liver lipid) after the stripping procedure. (B) Incorporation of ^{125}I into the $\beta\gamma_2$ complex (closed squares) or into the α chain (open squares) relative to the IgE. (C) Ratio of counts in β to those in α chains from receptor biosynthetically labeled with [^{35}S]methionine. [The γ chains do not incorporate methionine (Kinet et al., 1983).]

able (a) to extrinsically label the α chain (above), thereby permitting us to analyze the dissociation by comparing the α:$\beta\gamma_2$ ratios, and (b) to isolate intact receptors in a simple solvent, i.e., submicellar detergent without added lipids. The same considerations have now permitted us to examine

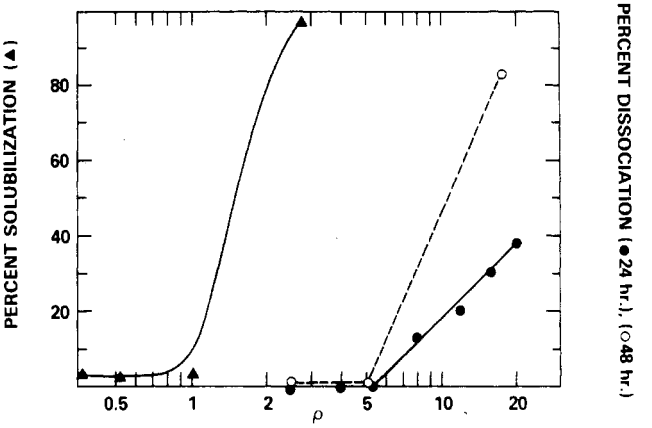

FIGURE 2: Effect of varying the ratio of detergent to phospholipid micelles (ρ) on the solubilization and dissociation of the receptor for IgE. Cells were reacted with monomeric iodinated IgE, washed, and then incubated with increasing concentrations of tumor-derived phospholipids in 10 mM CHAPS. The percentage of receptors solubilized (▲) was calculated from the counts in the supernatant after a high-speed centrifugation, compared to the total cell-bound counts. Two microliters of purified IgE–receptor complexes were incubated at 4 °C for 24 h (closed circles) or 48 h (open circles) with 200 μL of increasing concentrations of tumor-derived phospholipids in 10 mM CHAPS. The complexes were immunoprecipitated with anti-IgE prior to analysis on gels. The radioactivity in the individual components was determined by densitometry of the radioautograph.

quantitatively the effect of lipids in preventing the dissociation of the receptor by micellar detergent.

Figure 2 shows that relatively small amounts of tumor lipids can suppress the dissociation of the receptor by micellar concentrations of the detergent CHAPS. The abscissa shows the values of ρ: the ratio of micellar detergent to micellar lipids (Rivnay & Metzger, 1982). It can be seen that although complete *solubilization* of the receptor occurs when this ratio is above 2, no *dissociation* of the receptor occurs until a ratio of greater than 5 is achieved even over prolonged periods of time.

We previously had some indication that whereas mixed phospholipids from the tumor cells could protect against the dissociation induced by detergent, several individual lipids were less effective (Rivnay et al., 1982). However, in the absence of a practical quantitative assay, it was difficult to extend those relatively qualitative observations. Table II summarizes our new results. It can be seen that only the complex mixtures of phospholipids from hepatic cells were as effective as the tumor-derived lipids in inhibiting the detergent-induced dissociation.

A possible explanation for the differences between the individual lipids we tested and the tumor-derived lipids was that the former could not interact as well with the micellar detergent. If so, then the effectiveness of the purified lipids to counteract the ability of the detergent to solubilize cells should be reduced likewise. The data in Figure 3 demonstrate that this is not the case. With the possible exception of phosphatidylethanolamine (open circles), each of the purified lipids was effective in preventing the solubilization of the receptor at a concentration of 5 mM or higher (ρ of $\leq$1).

The ability of lipids to protect against dissociation is also seen with detergents other than CHAPS. Table III shows that although the protective effect is variable, it is substantial for both Triton X-100 and octyl glucoside. We previously showed that under less disruptive conditions (4 °C, 4 h) the variable dissociation induced by different detergents (in the absence of lipids) was accounted for by normalizing the data to equivalent concentrations of micellar detergent [see Figure 3A

Table II: Dissociation of Receptor in 10 mM CHAPS/Lipid Mixtures

type of lipid (2 mM)	incubation temp (°C)	incubation time (h)	% dissociation ± SD
unfractionated phospholipids			
tumor or liver	4	72	0
tumor	25	12	0
soybean	4	48	48
fractionated lipids			
PS, PE, PC, PI	25	6	57 ± 10
SM	25	6	33 ± 13
DG	25	6	0
	25	12	50 ± 6
cholesterol	25	6	0
	25	12	42 ± 2
mixtures of fractionated lipids[a]			
ternary mixtures of PS, PE, PC, PI	25	6	48 ± 11
other			
PS + PE + PC + PI	25	6	52 ± 6
PS + PE + PC + SM	25	6	52 ± 6
PS + PE + PC + PI + DG	25	6	52 ± 6

[a] For the mixtures, the individual lipids were added in equimolar amounts. The final concentration of total added lipids in the incubation was 2 mM. Avanti Corp. kindly provided us with an analysis of the fatty acid side chains associated with the different phospholipids. They varied widely. For example, the phosphatidylethanolamine contained approximately 23% each of palmityl, stearyl, and arachadonyl fatty acids, whereas the corresponding values for phosphatidylserine and phosphatidylinositol were 1–2%, 43%, and 1%, respectively. Studies with other systems suggested that in general the principal differences between the interactions of specific phospholipids and membrane proteins are related to the head groups and not the fatty acid side chains (Sanderman, 1978).

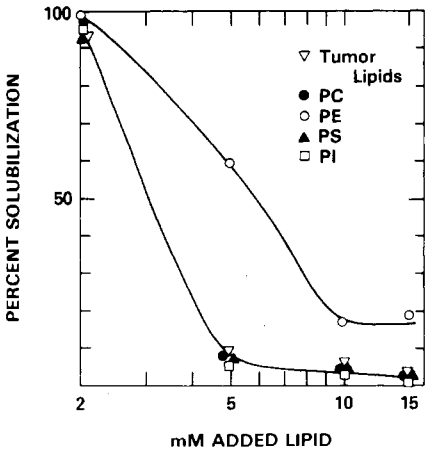

FIGURE 3: Inhibition of detergent-mediated solubilization of the receptor for IgE by added phospholipids. Cells were incubated with ^{125}I-IgE to fill the receptors (Kulczycki & Metzger, 1974) and then washed. Twenty-microliter aliquots, containing 1×10^5 cells, were added to 480 μL of pH 7.4 phosphate-buffered NaCl containing 5% fetal calf serum, 10 mM CHAPS, and 0–15 mM phospholipid. After incubation at 4 °C for 30 min, the mixture was centrifuged and the supernatant counted for released counts. Since each cell contains about 4×10^{-2} pmol of phospholipid (Rivnay & Metzger, 1982), the cells in the final incubation mixture maximally contributed 8 μM mixed phospholipids to the total.

Table III: Dissociation of the Receptor for IgE by Different Detergents in the Presence and Absence of Lipids[a]

detergent type	detergent concn (mM)[b]	tumor lipids (2 mM)	% dissociation
CHAPS	10	–	57 (±3)
CHAPS	10	+	"0"
Triton X-100	5.4	–	96 (±1)
Triton X-100	5.4	+	45
octyl glucoside	24.5	–	96 (±2)
octyl glucoside	24.5	+	74 (±1)

[a] The receptor was incubated for 12 h at room temperature prior to analysis. The dissociation was assessed by analyzing the ratio of counts in α to those in β plus γ by densitometry of autoradiographs of gels on which the receptor preparations were analyzed. The values in parentheses are the range observed in duplicate samples. [b] The total concentration of detergent was such that the concentration of *micellar* detergent would be approximately 5 mM in each case.

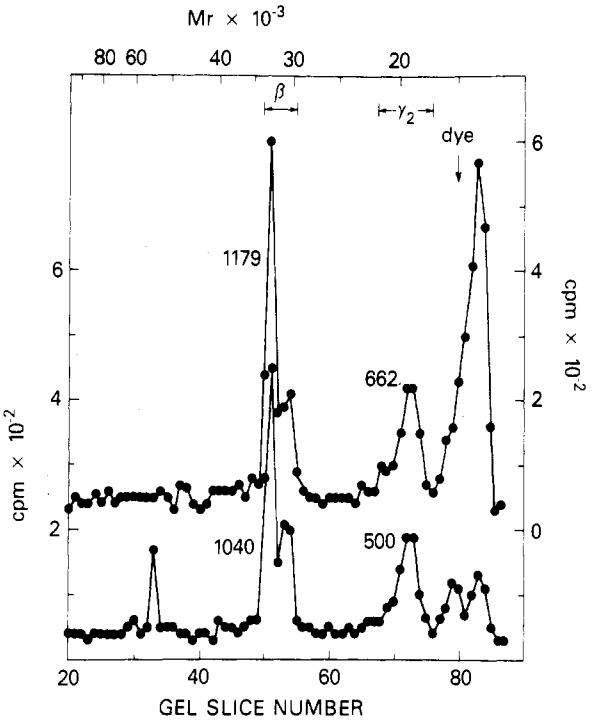

FIGURE 4: Incorporation of [^{3}H]palmitate into the subunits of the receptor on cells preloaded or not preloaded with IgE. The figure shows the counts of tritium in the slices from a 12.5% gel. The (unreduced) ^{125}I-IgE was in slices 7–11, and these contained 4630 and 4510 cpm in the upper and lower patterns, respectively. The remaining slices contained 100 or less cpm/slice of ^{125}I. The numbers next to the β and γ_2 components are the total counts in the peaks after subtraction of the ~50 cpm background/slice and a ~3% correction for the difference in recovered counts in IgE. Upper pattern, cells incubated with palmitate before addition of IgE; lower pattern, cells loaded with IgE prior to incubation with palmitate.

in Kinet et al., 1985)]. The concentrations of detergents compared in Table III were such that by using the critical micelle concentration determined for the detergents at 4 °C (Kinet et al., 1985), equivalent concentrations of micellar detergent should have been present (~5 mM). It is likely that at 25 °C the concentrations of micelles would have been somewhat higher. Nevertheless, given the data in Figure 2, one would still have expected the lipids to protect more completely since it is unlikely that a ρ value of ~5 would have been exceeded in any of the incubations. That the lipids were less effective in counteracting the effects of Triton X-100 and octyl glucoside emphasizes the complexity of the interactions even in the relatively simple mixtures of receptor, lipid, and detergent that we are analyzing. It is probably not coincidental that the ability to reincorporate IgE–receptor complexes into liposomes was also more efficient with the detergent CHAPS than with octyl glucoside (Rivnay & Metzger, 1982).

Covalently Bound Lipid. When the cells are grown in a medium containing tritiated palmitate, the fatty acid is rapidly taken up by the cells, with ~75% of the counts becoming cell-bound in ~4 h (data from five experiments). The receptor

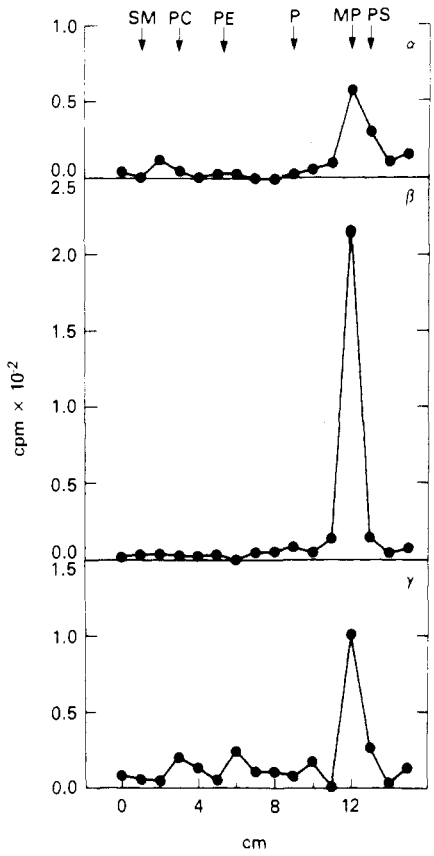

FIGURE 5: Analysis by thin-layer chromatography of tritiated residues released from subunits of the receptor. Receptors from cells incubated with [^{3}H]palmitate were purified and analyzed on gels to separate the subunits. The gels were sliced on the basis of an autoradiogram and the slices extracted with methanolic KOH. The extracts were then run on thin-layer chromatography and the sheets sliced and counted for radioactivity. The standards are SM, PC, PE, P (palmitic acid), PM (methyl palmitate), and PS.

for IgE isolated from such cells reproducibly showed counts associated with the β and γ subunits but few with the α chains (Figure 4).

Altogether, the total counts that were incorporated were small so that our analyses had to be limited. Nevertheless, a number of observations have been made. (a) When purified with submicellar detergent, the preparations of receptor when electrophoresed on gels reveal a labeled band of lipids that migrates just ahead of the dye front (Figure 4). This is consistent with the presence of bound lipid which we have postulated to be associated with the receptor (above and Discussion). If the immune precipitates of such preparations are rigorously extracted with organic solvents (Materials and Methods), over 99% of these noncovalently bound lipids are removed, but the majority of the counts associated with the β and γ chains remain (Table IV). (b) If the gels are treated with methanolic KOH, a treatment known to cleave fatty acid esterified to peptides, and the radioactivity is analyzed by thin-layer chromatography with several standards, virtually all of the counts are associated with a band that has the same R_f as methyl palmitate (Figure 5). (c) If the cells are grown in the presence of either tritiated myristic acid or tritiated oleic acid, radioactivity is similarly seen at the positions of the β and γ chains and also more prominently with the α chains. (Table IV). (d) The incorporation of counts in β and γ chains is quantitatively similar if cells are grown in radioactive palmitate before or after addition of IgE (Figure 4). We have documented elsewhere that β and γ chains do not turn over independently of α (Quarto et al., 1985). Therefore, modification of the β and γ chains on receptors that had already bound IgE indicates that the esterification occurs after the receptor was inserted into the plasma membrane. The results also imply that the binding of IgE to the receptor does not substantially influence the rate or extent of modification of the chains.

We had originally hoped to explore whether aggregation of the receptors, which initiates exocytosis, would influence the modification of the receptor of fatty acids. Regrettably, the incorporation of radioactive fatty acids—with or without aggregation of the receptors—was so small that a careful study of this question was impractical.

Discussion

In a protein that normally exists in an aqueous environment, a substantial proportion (60%) of the hydrophobic residues are buried in the protein's interior where they can interact with each other but not with solvent. [For a recent discussion, see Dill (1985).] On the other hand, the hydrophobic residues in those segments of membrane proteins that have substantial contact with the lipid bilayer can interact with the lipid moieties there. Of particular interest are the stability and specificity of such interactions [e.g., see Esmann et al. (1985)] and the role these play in the native conformation of the protein.

Our data on the receptor for IgE suggest that this membrane protein interacts with lipids both covalently and noncovalently. The noncovalently bound lipid can in turn be classified into two broad categories—weakly bound and tightly bound—strictly on the basis of operational criteria. The weakly bound lipid is that which appears to be removed by very brief exposure to small amounts of micellar detergent; the tightly bound lipid is that lipid which can be removed only by exposing the receptor to large amounts of detergent over prolonged periods of time.

The presence of weakly bound lipids provides the simplest explanation for the failure of the α chain in either the IgE–α-chain complex or the IgE–$\alpha\beta\gamma_2$ complex to become extrinsically iodinated. When such complexes are briefly exposed to micellar detergent (without lipid) and then maintained in submicellar detergent, the α chain becomes susceptible to iodination. Examination of the eluate from such a stripping wash of column-bound receptors indicates the presence of phospholipids (R. Quarto, unpublished observations), but these have not been analyzed. Furthermore, if micellar detergent with or without lipids is added back to such susceptible IgE–receptor complexes, iodinatability is once more suppressed. This indicates that the interaction of α with lipids is likely to be rather nonspecific since the lipids can be replaced by a variety of detergents to produce the same effect. Whether the iodination of α is prevented by the presence of lipids (or micellar detergent) for steric reasons or indirectly because of conformational changes cannot of course be determined from our data. After the stripping procedure, the interaction between the α chain and IgE, and between the α chain and the β chain and two γ chains, is preserved, even when neither detergent nor lipids are restored (Figure 1). It is likely, therefore, that at least the loosely bound lipid is not critical for maintaining the basic conformation of α.

Our data suggest that, in addition, the receptor interacts more strongly and specifically with lipids and that these interactions are required to maintain the subunit structure of the receptor. We previously showed that exposure of the receptor to a variety of micellar detergents led to progressive dissociation of the subunits of the receptor. The detergent micelle:receptor ratios that were required to dissociate the

Table IV: Incorporation of Fatty Acids into the Subunits of the Receptor for IgE[a]

rel incorpn/mol of subunit[b]	labeled fatty acid					
	myristic		oleic		palmitic	
	not extracted	extracted	not extracted	extracted	not extracted	extracted
α	0.41	0.55	0.62	0.63	0.31	0.43
β	"1"	0.78	"1"	0.93	"1"	1.32
γ	0.39	0.28	0.46	0.54	0.80	0.72
phospholipid	75	0.048	167	0.31	78	0.01

[a] After being grown in labeled fatty acids, the cells were reacted with ^{125}I-IgE, washed, and solubilized, the IgE–receptor complexes were purified on columns and then immunoprecipitated in duplicate. One each of the precipitates was extracted with chloroform/methanol and ether/ethanol (Materials and Methods), and then the precipitates were electrophoresed on gels. After radioautography, the gels were sliced into 2-mm slices and counted in a scintillation counter. [b] For each fatty acid, the counts in each of the subunits and in the phospholipids were first normalized on the basis of the ^{125}I-IgE in the gel and then further normalized on the basis of the counts in the β subunit of the *unextracted* precipitates. For myristic, oleic, and palmitic acids, the absolute counts in β of the latter precipitates were 1467, 446, and 1771, respectively, and after normalization for the IgE, 1467, 416, and 1535, respectively.

subunits were 5 orders of magnitude higher than the detergent:lipid ratios required to solubilize membranes, and the rate of dissociation was much slower (Kinet et al., 1985). Furthermore, we had evidence that the dissociation involved an equilibrium between the detergent, the receptor protein, and a third component. Our previous qualitative observations (Rivnay et al., 1982) and our present quantitative analyses strongly suggest that this third component is tightly bound lipid. Only mixed-membrane phospholipids are able to optimally protect against dissociation (Table II) whereas a variety of discrete lipids are able to counteract the detergent-induced solubilization of cells (Figure 3). This is evidence for the proposal that the protective effect of the lipid results from specific interactions of lipid with the receptor and not simply from "neutralization" of the detergent by the added lipid.

We could explore further the specificities of the interactions of both the weakly and tightly bound lipids by more extensive experiments like those described in Table II. However such studies would simply accumulate more indirect data and could never by themselves be entirely convincing. This is especially true because we are only able to examine a rather gross criterion of the integrity of the receptor, i.e., subunit association. In the absence of more sensitive functional criteria, a more informative and rigorous approach will be to analyze directly the lipids associated with the receptor. The purification and iodination protocols we have devised should allow one to quantitate the recovery of the intact receptor accurately and to examine separately the more loosely and firmly bound lipids. Our former colleague B. Rivnay has developed analytical procedures of the requisite sensitivity for such an analysis (Rivnay, 1985) and is attempting this direct approach. This direct approach may also permit a more complete assessment of the covalently bound lipid. Although our present data provide substantial evidence that the chains have ester-linked fatty acid, the results are largely qualitative. The incorporation of label is too low to make a quantitative analysis practical, and compositional analysis may be more informative. A demonstration that a substantial proportion of the receptors contains esterified lipids is a necessary—though of course not sufficient—criteria for postulating a functional role for such moieties.

The proximal biochemical events that accompany aggregation of the receptor for IgE and the role of the receptor in these are still uncertain. An important tool for elucidating such events will be to search for activities of purified receptors and to reconstitute the receptor in artificial systems. Quantitative comparisons of perturbations induced by aggregating the receptor in such systems with the early events on intact cells and membranes will be essential. By analogy with other systems, the lipid composition of such reconstituted systems may be important [e.g., see Kiribusky & Schramm (1983) and Esmann et al. (1985)]. Our current observations should be helpful in permitting further analysis of the receptor so that such reconstitutions can optimally mimic the milieu in vivo.

References

Alcaraz, G., Kinet, J.-P., Kumar, N., Wank, S. A., & Metzger, H. (1984) *J. Biol. Chem. 259*, 14922–14927.

Barsumian, E. L., Isersky, C., Petrino, M. G., & Siraganian, R. P. (1981) *Eur. J. Immunol. 11*, 317–323.

Conrad, D. H., & Froese, A. (1976) *J. Immunol. 116*, 319–326.

Dill, K. A. (1985) *Biochemistry 24*, 1501–1505.

Eccleston, E., Leonard, B. J., Love, J. S., & Welford, H. J. (1973) *Nature (London), New Biol. 244*, 73–76.

Esmann, M., Watts, A., & Marsh, D. (1985) *Biochemistry 24*, 1386–1393.

Holowka, D., & Metzger, H. (1982) *Mol. Immunol. 19*, 219–227.

Holowka, D., Hartmann, H., Kanellopoulos, J., & Metzger, H. (1980) *J. Receptor Res. 1*, 41–68.

Kanellopoulos, J., Rossi, G., & Metzger, H. (1979) *J. Biol. Chem. 254*, 7691–7697.

Kanellopoulos, J. M., Liu, T. Y., Poy, G., & Metzger, H. (1980) *J. Biol. Chem. 225*, 9060–9066.

Kinet, J.-P., Perez-Montfort, R., & Metzger, H. (1983) *Biochemistry 22*, 5729–5732.

Kinet, J.-P., Alcaraz, G., Leonard, A., Wank, S., & Metzger, H. (1985) *Biochemistry 24*, 4117–4124.

Kiribusky, J., & Schramm, M. (1983) *J. Biol. Chem. 258*, 6841–6849.

Liu, F.-T., Bohn, J. W., Ferry, E. L., Yamamoto, H., Molinaro, C. A., Sherman, L. A., Klinman, N. R., & Katz, D. H. (1980) *J. Immunol. 124*, 2728–2736.

McConahey, P. J., & Dixon, F. J. (1966) *Int. Arch. Allergy Appl. Immunol. 29*, 185–189.

Metzger, H., Kinet, J.-P., Perez-Montfort, R., Rivnay, B., & Wank, S. A. (1983) in *Progress in Immunology* (Yamamura, Y., & Tada, T., Eds.) Vol. 5, pp 493–501, Academic Press, Orlando, FL.

Pecoud, A. R., & Conrad, D. H. (1981) *J. Immunol. 127*, 2208–2213.

Perez-Montfort, R., Kinet, J.-P., & Metzger, H. (1983) *Biochemistry 22*, 5722–5728.

Quarto, R., Kinet, J.-P., & Metzger, H. (1985) *Mol. Immunol. 294*, 303–315.

Rivnay, B. (1985) J. Chromatogr. 294, 303-315.
Rivnay, B., & Metzger, H. (1982) J. Biol. Chem. 257, 12800-12818.
Rivnay, B., Wank, S. A., & Metzger, H. (1982) Biochemistry 21, 6922-6927.
Rossi, G., Newman, S. A., & Metzger, H. (1977) J. Biol. Chem. 252, 704-711.
Sandermann, H. (1978) Biochim. Biophys. Acta 515, 209-237.
Schmidt, M. F. G., Bracha, M., & Schlessinger, M. J. (1979) Proc. Natl. Acad. Sci. U.S.A. 76, 1687-1691.

Characterization of Glucocorticoid Receptor in HeLa-S3 Cells†

H. Hoschützky and O. Pongs*
Lehrstuhl für Biochemie, Ruhr-Universität Bochum, 4630 Bochum-Querenburg, FRG
Received March 20, 1985

ABSTRACT: Glucocorticoid receptor of the human cell line HeLa-S3 has been characterized and has been compared to rat and to mouse glucocorticoid receptors. If HeLa cells were lysed in the absence of glucocorticoid, glucocorticoid receptor was isolated in a nonactivated form, which did not bind to DNA–cellulose. If HeLa cells were preincubated with glucocorticoid, glucocorticoid receptor was isolated in an activated, DNA-binding form. HeLa cell glucocorticoid receptor bound [^{3}H]triamcinolone acetonide with a dissociation constant (K_D = 1.3 nM at 0 °C) that was similar to those of mouse and rat glucocorticoid receptors. Similarly, the relative binding affinities for steroid hormones decreased in the order of triamcinolone acetonide > dexamethasone > promegestone > methyltrienolone > aldosterone ≥ moxestrol. Nonactivated and activated receptors were characterized by high-resolution anion-exchange chromatography (FPLC), DNA–cellulose chromatography, and sucrose gradient centrifugation. Human, mouse, and rat nonactivated glucocorticoid receptors had very similar ionic and sedimentation properties. Activated glucocorticoid receptors were eluted at similar salt concentrations from DNA–cellulose columns but at different salt concentrations from the FPLC column. A monoclonal mouse anti-rat liver glucocorticoid receptor antibody [Westphal, H. M., Mugele, K., Beato, M., & Gehring, U. (1984) *EMBO J. 3*, 1493-1498] did not cross-react with HeLa cell glucocorticoid receptor. Glucocorticoid receptors of HeLa, HTC, and S49.1 cells were affinity labeled with [^{3}H]dexamethasone and with [^{3}H]dexamethasone 21-mesylate. The molecular weights of [^{3}H]dexamethasone 21-mesylate labeled glucocorticoid receptors (M_T 96 000 ± 1000) were undistinguishable by polyacrylamide gel electrophoresis. Protease digestions of [^{3}H]dexamethasone 21-mesylate labeled HeLa cell and HTC cell glucocorticoid receptor by α-chymotrypsin and by *Staphylococcus aureus* V8 protease revealed structural differences between human and rat glucocorticoid receptor. Since the steroid-binding and the DNA-binding domains were apparently similar in human and rat glucocorticoid receptor, the differences in FPLC, antibody binding, and protease digestion patterns probably reflect structural differences in the immunogenic domains of human and rat glucocorticoid receptor.

Glucocorticoid receptors (GR) of various tissues and species, especially of rat liver, have extensively been characterized [for recent reviews, see Carlstedt-Duke et al. (1983), Schmidt & Litwack (1982), and Gustafsson et al. (1984)]. GRs are apparently the mediator of glucocorticoid action, which eventually results in the selective activation/repression of the expression of various genes. The extensive studies of isolating and purifying GRs have resulted in a complex, sometimes even confusing, picture of GR properties (Koblinsky et al., 1972; Yamamoto et al., 1976; Eisen & Glinsman, 1978; Litwack et al., 1978; Govindan & Gronemeyer, 1984; Wrange et al., 1979; Sherman et al., 1983). GRs have been isolated in the form of several isotypes, which were characterized by their abilities to interact with hormone or with DNA, respectively (Higgins et al., 1973; Grippo et al., 1983). These studies were usually interpreted in support of the common paradigm of steroid action (Gorski et al., 1968; Jensen et al., 1968), which postulated an interaction of GR with glucocorticoid in the cytosol prior to its possible interaction with chromatin in the nucleus. It was attempted to assign these isotypes to "cytosolic" and to "nuclear" variants of GR. Accordingly, GR has been localized immunocytochemically in the nuclei and cytoplasm of target cells (Antakly & Eisen, 1984). However, recent papers (Welshons et al., 1984; King & Greene, 1984, Gasc et al., 1984) about the cellular locations of estrogen and progesterone receptors have cast some doubt on the general significance of receptor isotypes produced by elaborate purification schemes in vitro.

Albeit the plethora of literature on GR of rat liver, little is known about the properties of human GR (Currie & Cidlowski, 1980; Kontula et al., 1981; Harmon et al., 1984; Eliard & Rousseau, 1984). Monoclonal antibodies, which were raised against rat liver GR, generally failed to cross-react with GR of human cells (Westpahl et al., 1982; Okret et al., 1984). One study with an immunoglobulin fraction of serum from a rabbit immunized with rat liver GR reported a cross-reaction to GR of human tissue (Okret et al., 1981). We have investigated the properties of GR from HeLa cell lysates. In this paper we report the general molecular properties of human GR. High-resolution anion-exchange chromatography (FPLC) as well as DNA–cellulose column chromatography were used to characterize various forms of human GR generated in HeLa cell lysates. The molecular properties of the steroid hormone

† This work was supported by grants from the Volkswagenstiftung and the Land Nordrhein-Westfalen to O.P.

0006-2960/85/0424-7348$01.50/0