Hydrodynamic Characterization of the Photoaffinity-Labeled Insulin Receptor Solubilized in Triton X-100†

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ABSTRACT: The insulin receptor in isolated rat liver plasma membranes was covalently labeled with the photoreactive insulin analogue N^{B-29}-[(4-azido-2-nitrophenyl)acetyl]insulin and solubilized with the nondenaturing detergent Triton X-100. The resulting protein–detergent complex was characterized by gel filtration on Sepharose 6B, sedimentation rate determination in linear sucrose gradients, and equilibrium isopycnic centrifugation in NaBr and CsCl. The labeled insulin receptor was found in two forms. The Stokes radii and $s_{20,w}$'s of the two receptor–detergent complexes (R1 and R2) were (mean ± SEM) 7.08 ± 0.04 and 3.62 ± 0.05 nm and 10.45 ± 0.04 and 6.54 ± 0.15 S, respectively. The two forms appeared to have the same buoyant density, 1.285 ± 0.002 g cm^{-3}. The dissociation of R2 from R1, or its reaggregation, either with itself or with other unlabeled proteins, to give R1 proceeded without chemical modification. Mild reduction of disulfide bonds (1 mM 1,4-dithiothreitol) increased the dissociation of R2 from R1. These results indicate that the solubilized receptor binds significant amounts of detergents, that the insulin binding component of the receptor binds to other receptor components by hydrophobic interactions, and that one or more components of the insulin receptor contain intrachain disulfide bonds.

The action of insulin on target cells is generally believed to be initiated by the binding of the hormone to a specific binding protein, known as the receptor, in the cell membrane. It is still not clear, however, how this binding elicits a response from the cytoplasm of the cell. Research has centered recently on the characterization of the components of the receptor in order to elucidate this mechanism. Several affinity-labeling techniques have been used to ascribe an apparent molecular weight of (1.3–1.4) × 10^5 to the insulin binding component (IBC) of the receptor by using NaDodSO$_4$–polyacrylamide gel electrophoresis[1] (Yip et al., 1978, 1980; Pilch & Czech, 1979, 1980; Jacobs et al., 1979; Wisher et al., 1980). We have shown that this is an overestimate due to differences in electrophoretic mobility between marker proteins used and the IBC, the true molecular weight being close to 9 × 10^4 (Wisher et al., 1980).

Studies of the Triton X-100 (TX100) solubilized receptor have shown it to be active, with essentially identical insulin binding kinetics with the membrance-located receptor. Molecular weight estimates [(0.3–1.0) × 10^6] are much larger than those from NaDodSO$_4$–polyacrylamide gel electrophoresis (Cuatrecasas, 1972; Ginsberg et al., 1976; Lang et al., 1980a). None of these studies, however, took into account the binding of TX100 to the protein, which for membrane proteins can be up to 1.46 g of detergent per g of protein (Helenius & Simons, 1975).

We report here the hydrodynamic characterization of the receptor–detergent complex, using radiolabeled photoreactive insulin analogues (photoprobes) to identify the receptor. Gel filtration, sedimentation velocity determination in sucrose gradients, and equilibrium isopycnic centrifugation were used to determine the size and weight of the receptor–detergent complex and estimate the amount of detergent bound.

Experimental Procedures

Materials. Calibrating proteins for gel filtrations and sucrose gradient centrifugation were from Sigma. Sepharose 6B was from Pharmacia. TX100 was scintillation grade from Packard. ^{125}I for iodination of proteins was from the Radiochemical Centre, Amersham. BSA was Cohn fraction V from Sigma. All other reagents were AnalaR grade.

Methods. Isolation of rat liver plasma membranes was by the method of Touster et al. (1970). Iodination of insulin and insulin analogues was essentially as previously described (Wisher et al., 1980). Membranes (2 mg of protein cm^{-3}) were incubated with photoprobe (5 mM) in 75 mM Tris-HCl, pH 7.8, containing 30 mM NaCl, 10 mM glucose, and 1% (w/v) bovine serum albumin. Nonspecific labeling was determined in parallel incubations containing 10^{-5} M pork insulin. Most of the experiments were carried out by using a new photoprobe, N^{B-29}-[(4-azido-2-nitrophenyl)acetyl]insulin, the synthesis and characterization of which have also been described (Thamm et al., 1980). Photoactivation time with this analogue was 5 min.

Solubilization of Membranes. Membranes were homogenized in 2% (v/v) TX100 (100 μL/mg of membrane protein), incubated for 30 min at 30 °C, and spun at 80000*g* for 30 min to remove nonsolubilized material. This procedure solubilized 60–80% of radioactivity in the original pellet. Increasing the amount of TX100 did not increase solubilization of radioactive-labeled material, and NaDodSO$_4$–polyacrylamide gel electrophoresis analysis of TX100-insoluble material revealed no specifically labeled bands.

Gel Filtration. Gel filtration was performed at 5 °C on a Sepharose 6B column (1.6 × 95 cm) eluted with 0.2% (w/v) (16.52 mM) Tris-HCl, pH 7.8, 0.1% (v/v) TX100, and 0.02% (w/v) NaN_3 (T/T buffer) at 5.55 mL h^{-1}; 1.85-mL fractions

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[1] Abbreviations used: NaDodSO$_4$–polyacrylamide gel electrophoresis, polyacrylamide gel electrophoresis in sodium dodecyl sulfate; TX100, Triton X-100; DTT, 1,4-dithiothreitol; cpm, counts per minute; BSA, bovine serum albumin; Tris-HCl, tris(hydroxymethyl)aminomethane hydrochloride.

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Table I: Calibrating Proteins for Gel Filtration and Sedimentation Velocity Analysis

protein	diffusion coefficient[a] ($\times 10^7$)	sedimentation coefficient ($\times 10^{13}$)	partial specific volume ($cm^3\ g^{-1}$)
thyroglobulin	2.5[e]	19.2[f]	0.72[f]
β-galactosidase	3.12[g]	15.9[g]	0.76[g]
urease	3.46[c]		
apoferritin	3.61[g]	17.6[g]	0.74[g]
catalase	4.1[c]	11.3[b]	0.73[c]
yeast alcohol DH	4.7[e]	7.4[b]	0.769[h]
ovalbumin	7.8[e]	3.55[f]	0.749[f]
[^{125}I]insulin	1.6[f]	1.2[f]	0.735[f]

[a] Stokes' radii were calculated from the relationship $a = kT/6\pi\eta D$ where a is the Stokes radius, k is the Boltzmann constant, T is the temperature in kelvins, η is the viscosity of the medium in poise, and D is the diffusion coefficient. Values of a for ferritin and ferritin dimer also used to calibrate the gel column were published values, 6.0[d] and 7.9,[b] respectively. [b] Siegel & Monty (1966). [c] Clark (1975). [d] Ackers (1964). [e] Andrews (1965). [f] Sober (1970). [g] Haga et al. (1977). [h] Martin & Ames (1961).

were collected. Calibration of the column with respect to Stokes' radius was by the method of Porath (1963) using the values listed in Table I. The void volume (V_0) was determined by using Blue Dextran 2000 (Pharmacia) and the liquid volume (V_l) was determined by using the elution volume of Na^+. This calibration has been shown to be linear for proteins of Stokes' radii from 2.4 to 10.7 nm and frictional ratios (f/f_0) from 1.14 to 2.34 (Siegel & Monty, 1966).

Sucrose Density Gradients. Linear gradients of 5–20% (w/w) sucrose in T/T buffer were poured and centrifuged as described by Martin & Ames (1961), using a Beckman SW50.1 rotor. Gradient volume was 5 cm^3, and samples of labeled material or marker proteins were applied in 20–100-μL volumes. Data were analyzed by using a trapezoidal approximation to the sedimentation integral (Martin & Ames, 1961), with 0.02-cm steps for ΔX, where X is the distance migrated by the particle; density and viscosity of the sucrose at each step were calculated by using the equations of Barber (1966). Comparison of the sedimentation of calibrating proteins in the presence and absence of 0.1% TX100 (v/v) in the gradients revealed no significant difference, indicating that changes in viscosity and density caused by the presence of detergent were within the error of the method. Values of $s_{20,w}$ for calibrating proteins calculated by this method were the following [mean ± SD (n)]: insulin, 1.42 ± 0.07 (8); ovalbumin, 3.63 ± 0.28 (5); catalase, 11.23 ± 0.19 (5). These compare well with the values given in the literature (Table I).

Equilibrium Density Gradients. Single step gradients of 6-cm^3 volume were run for 18 h in a Beckman 50 Ti rotor at 45 000 rpm and either 20 or 25 °C. The bottom layer (3 cm^3) was 50% (w/v) of the salt used in T/T buffer, and the concentration in the upper layer was adjusted to give a final mean concentration that would result in the density range required. Under these conditions, CsCl gradients achieve equilibrium in 12 h (Brunk & Leick, 1969). Comparison of the density gradients formed by NaBr with the theoretical equilibrium gradients showed that NaBr gradients also reached equilibrium in less than 18 h.

Density gradients were determined by measuring the refractive index of fractions from parallel blank gradients (no radiolabeled material). Refractive indexes were measured on a water-jacketed Abbé's refractometer; equations relating refractive index to density were from Birnie (1978), and values of the density gradient proportionality constant for calculation of equilibrium gradients were from Ifft et al. (1970).

Solubilized Receptor. Solubilized insulin receptor was assayed by the method of Ginsberg et al. (1978), modified by incubating the receptor for 2 h at 20 °C and washing the filtered precipitate with 1% (w/v) BSA in 0.1 M phosphate buffer, pH 7.4.

Protein Assays. Alcohol dehydrogenase was assayed by the method of Martin & Ames (1961) and catalase and β-galactosidase were assayed by the method of Clark (1975). Other proteins were measured by the method of Lowry et al. (1951), modified for the presence of TX100 (Dulley & Grieve, 1975).

Ultrafiltration. Samples were concentrated by ultrafiltration in Amicon cells using DM5000 or YM5000 membranes.

Calculation of Molecular Weight and Detergent Binding to the Receptor. For a multicomponent system, the partial specific volume ($\bar{v}$) of a complex of two or more components of that system can be closely approximated by $1/\rho$, where ρ is the buoyant density of the complex (Eason & Campbell, 1978). By use of the value of $\bar{v}$ calculated in this way, the molecular weight (M_r) and frictional ratio (f/f_0) of the complex can be determined from eq 1 and 2 (Siegel & Monty, 1966) where η is the viscosity and ρ_0 the density of the medium,

$$M_r = 6\pi\eta Nas/(1 - \bar{v}\rho_0) \quad (1)$$

$$f/f_0 = a/(3\bar{v}M/4\eta N)^{1/3} \quad (2)$$

N is Avogardro's number, a is the Stokes radius, and s is the sedimentation coefficient. Determination of the protein content of the complex requires that the partial specific volume of the protein ($\bar{v}_p$) and the amount of any components other than protein and detergent be known. Lipid is the most likely additional component for solubilized membrane protein, but TX100 at the concentrations used displaces almost all lipid from the protein (Helenius & Simons, 1975). The insulin receptor is thought to be a glycoprotein since it is bound to and activated by concanavalin A and other lectins (Cuatrecasas & Tell, 1973; Caron et al., 1978), and the best estimator of $\bar{v}_p$ for the receptor is therefore that of another membrane glycoprotein, such as γ-glutamyltransferase ($\bar{v}_p = 0.71$; Hughey & Curthoys, 1976), or band III glycoprotein from erythrocytes [$\bar{v}_p = 0.73$; calculated from data of Clark (1975)].

The amount of detergent bound can be determined from (Tanford et al., 1974)

$$\bar{v}^* = \frac{\bar{v}_p + \delta_D\bar{v}_D}{1 + \delta_D} \quad (3)$$

where $\bar{v}^*$ and $\bar{v}_D$ are the partial specific volumes of complex and detergent, respectively, and δ_D is the amount of detergent bound in grams per gram of protein. $\bar{v}_D$ was assumed to be the same for detergent bound to protein as for detergent in the micellar state; for TX100, $\bar{v}_D = 0.908\ cm^3\ g^{-1}$ (Tanford et al., 1974).

Results

Gel Filtration. Gel filtration on Sepharose 6B showed five peaks of labeled material (Figure 1). Peaks a, d, and e correspond to V_0, unbound photoprobe, and V_i, respectively. Insulin binding activity in unlabeled membranes coeluted with peak b. Of the radioactivity in peaks b and c, 86% was recovered in peak b (R1); 82% of this represented specific labeling. Specific labeling of peak c (R2) was only 56%. Peak c corresponded to the elution position of TX100 micelles, as determined by absorbance at 280 nm and the elution of [^{14}C]palmitate. Incorporation of photoprobe, or photo-

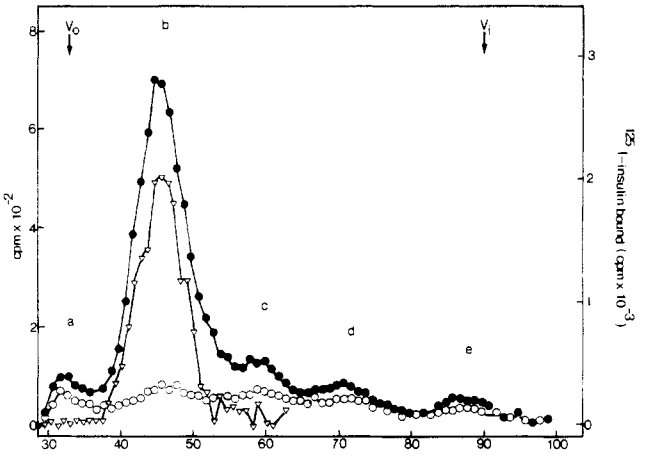

FIGURE 1: Gel filtration chromatography of TX100-solubilized rat liver plasma membranes. Membranes were labeled with $N^{B\text{-}29}$-[(4-azido-2-nitrophenyl)acetyl][^{125}I]insulin as described under Experimental Procedures. Insulin binding was measured in solubilized membranes that had not been labeled with photoprobe. Nonspecific binding to the receptor ([^{125}I]insulin bound in the presence of 3.2 μg cm^{-3} unlabeled insulin) was subtracted from total binding for each point. (●) Total labeling of membranes with photoprobe; (○) nonspecific labeling; (▽) specific binding of [^{125}I]insulin.

probe-labeled membrane lipid, into these micelles may account for the high percentage of nonspecific counts. Similar partial specificity of labeling was found by Sahyoun et al. (1978) by using glutaraldehyde cross-linking of [^{125}I]insulin to rat liver plasma membranes. The Stokes radii of R1 and R2 were determined to be [mean ± SEM (n)] 7.08 ± 0.04 (16) and 3.62 ± 0.05 nm (17), respectively.

Sucrose Density Gradients. Solubilized photoprobe-labeled membranes showed three peaks of specifically labeled material on sucrose gradients (Figure 2a), one of which comigrated with [^{125}I]insulin. Standard proteins showed a linear relationship between distance migrated into the gradient and $s_{20,w}$, and from this calibration curve, values of $s^{0.74}_{20,w}$ of 10.15 and 6.20 S were obtained for the other two peaks (average $\bar{v}$ for marker proteins = 0.74 cm^3 g^{-1}).

For determination of whether these two peaks corresponded to R1 and R2, samples of R1 and R2 from gel columns were concentrated and analyzed on sucrose gradients. Figure 2b,c shows that in each case both 10.15S and 6.2S components were seen, although R1 gave more 10.15 S than 6.2 S. R2 also contained a large amount of slowly sedimenting material which was absent from R1 and only partially specific. This probably reflects the heterogeneous nature of material of 3–4-nm Stokes' radius in the crude solubilized membrane preparation.

Effects of Reduction. Figure 3 shows the effect of reduction with DTT on the receptor analyzed by gel chromatography and sucrose gradients. The loss of R1 is paralleled by the disappearance of the 10.15S form of the receptor, with a simultaneous increase in R2 and 6.2S forms. These results indicate that the 10.15S form is equivalent to R1 and the 6.2S form to R2.

DTT will also reduce cysteine residues in the photoprobe, releasing the A chain, which is not covalently linked to the receptor. Sulfitolysis of the labeled photoprobe showed that 65–70% of the ^{125}I was incorporated into the A chain; the increase in very slowly sedimenting material after reduction is probably this free A chain. However, the increase in R2 was also observed in a similar experiment to Figure 3a by using [A-1-[γ-[(4-azidophenyl)acetyl]-D-α,γ-diaminobutyryl]]-insulin, in which the photoreactive group is on the A chain, and 80–90% of ^{125}I is incorporated into the A chain. Therefore, the changes in elution and sedimentation positions are

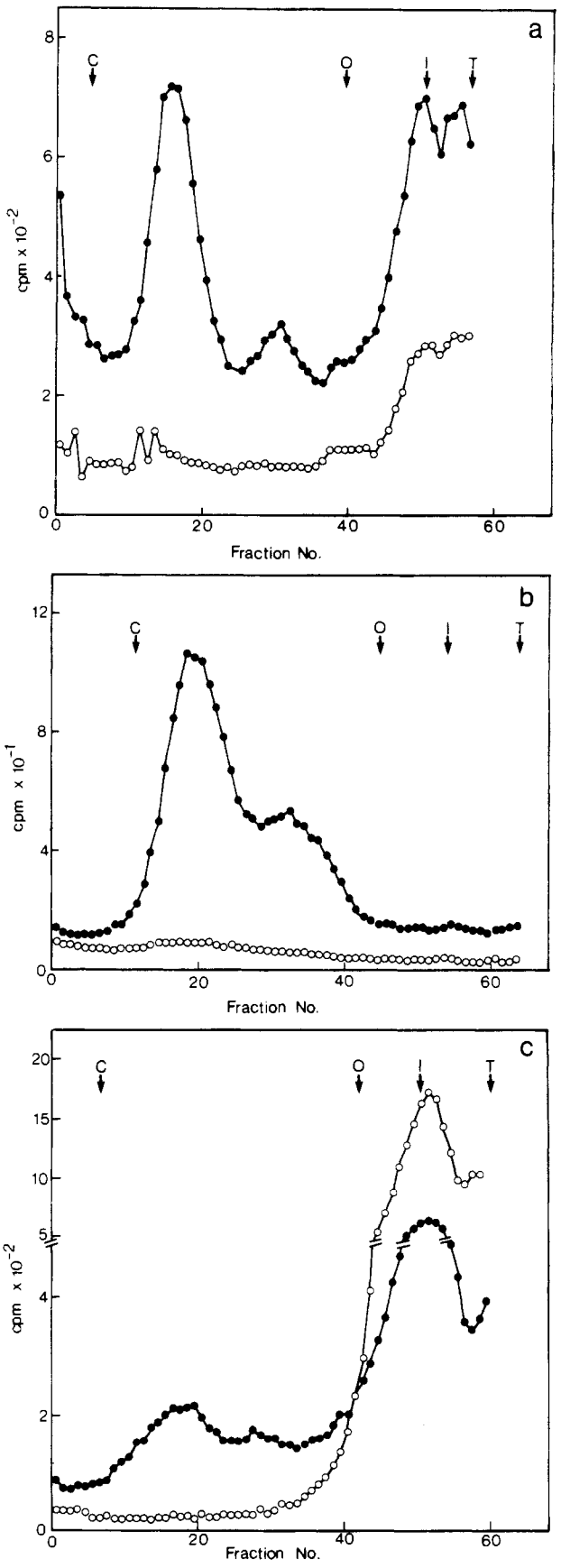

FIGURE 2: Sucrose density gradient analysis of ^{125}I-photoprobe-labeled, TX100-solubilized membranes. Gradients were centrifuged for 6 h at 45 000 rpm. (a) Unfractionated membranes. (b) Fractions 43–48 from Sepharose 6B columns pooled and concentrated. (c) Fractions 57–63 from Sepharose 6B columns pooled and concentrated. (●) Total labeling; (○) nonspecific labeling. The positions of marker proteins after centrifugation are indicated: C, catalase; O, ovalbumin; I, [^{125}I]insulin; T, top of gradient.

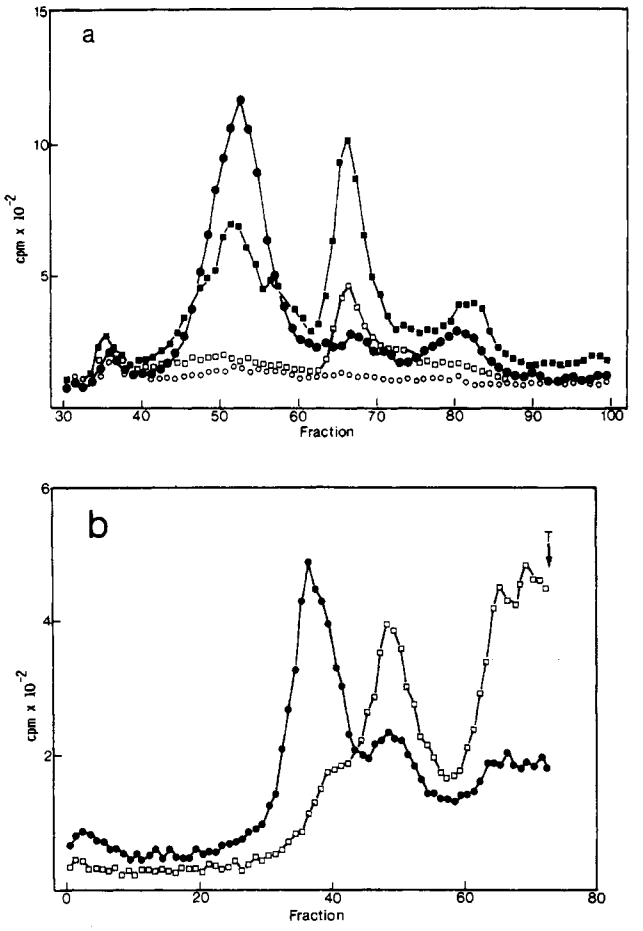

FIGURE 3: (a) Gel filtration of ^{125}I-photoprobe-labeled membranes. (■, ●) Total labeling; (□, ○) nonspecific labeling. Solubilized, labeled membranes were incubated for 30 min at 30 °C in the presence (■, □) or absence (●, ○) of 5 mM DTT and chromatographed with or without 1 mM DTT in the elution buffer. (b) Sucrose density gradient analysis of ^{125}I-photoprobe-labeled membranes. Solubilized, labeled membranes were incubated as (a) in the presence (●) or absence (□) of 1 mM DTT, and 100-μL samples were layered onto sucrose gradients. Reduced samples were analyzed on gradients containing 1 mM DTT. T, top of gradient.

not due to the separation of radioactive label from the receptor by thiol reduction.

Equilibrium Density Gradient Centrifugation. The solubilization of integral membrane proteins such as the insulin receptor (Cuatrecasas, 1972) involves the binding of detergent to the hydrophobic regions of the protein normally associated with lipid when the protein is in the membrane [for review, see Helenius & Simons (1975)]. The amount of detergent attached will depend upon the extent to which the protein is inserted into the membrane and will affect its apparent size in solution (Stokes' radius) and its density, and, hence, its sedimentation coefficient.

Analysis of TX100-solubilized, photoprobe-labeled membranes in CsCl gradients showed two bands of labeled material, plus material of density <1.2 g cm^{-3} which was probably labeled membrane lipid (Figure 4). The labeling of only one of these bands, however, was abolished by an excess of cold insulin. This material had a density of 1.32 ± 0.01 g cm^{-3}.

For minimization of the possible effects of high salt concentration on hydration and detergent binding, the density of this material was also studied in NaBr gradients. NaBr solutions have a higher water activity than CsCl and form shallower gradients, allowing more accurate determinations of the buoyant density of the detergent–receptor complex. Under such conditions, the labeled receptor had a density of

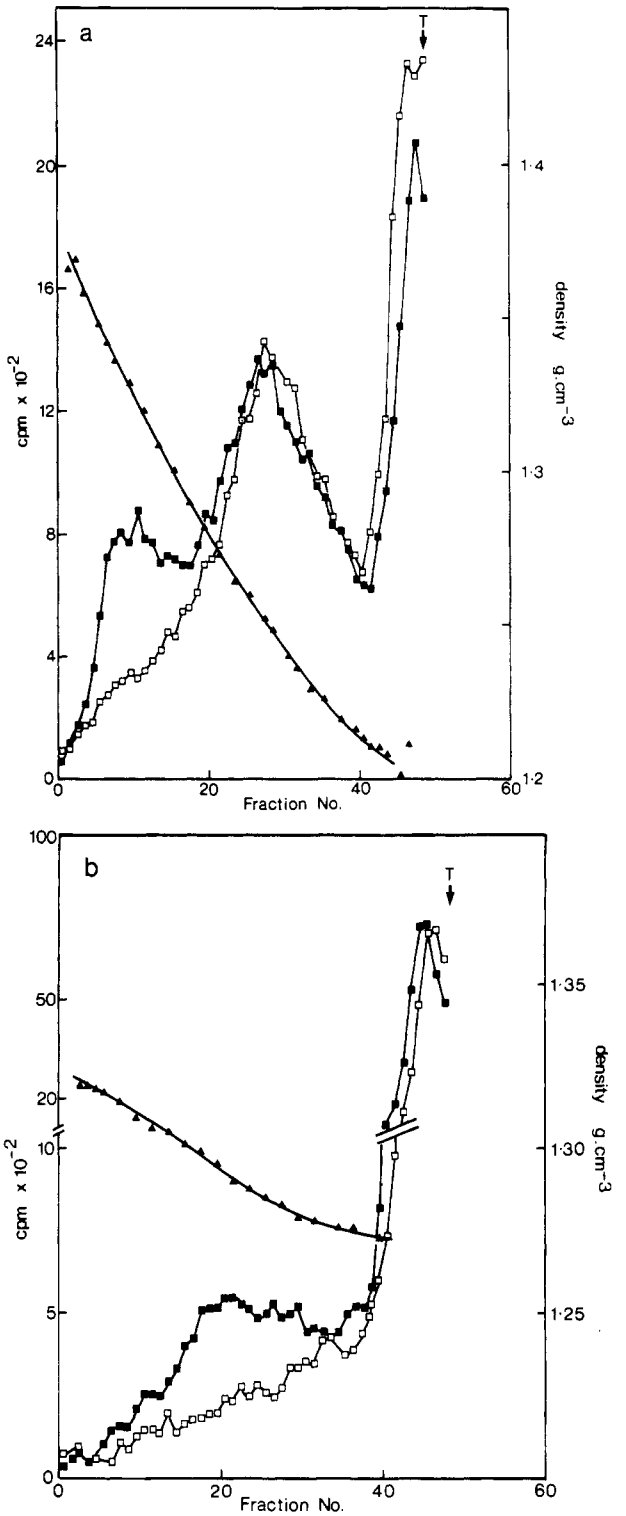

FIGURE 4: Isopycnic centrifugation of ^{125}I-photoprobe-labeled membranes. (a) CsCl gradient, mean concentration 35.8%. (b) NaBr gradient, mean concentration 41%. (■) Total labeling; (□) nonspecific labeling; (▲) density. T, top of gradient.

1.285 ± 0.002 (6) g cm^{-3}. The higher density in CsCl can be attributed to its lower water activity (Birnie, 1978). This density was then used to calculate $s_{20,w}$ for the protein–detergent complexes observed on sucrose density gradients, 10.45 ± 0.04 (8) and 6.54 ± 0.15 S (10).

Molecular Weights (M_r) and Detergent Binding to Receptors (δ_D). By use of eq 1, values for M_r*, the apparent molecular weights of the receptor–detergent complexes, were calculated to be 3.77 × 10^5 and 1.21 × 10^5 for R1 and R2, respectively. (The ranges for these molecular weights, based

on the 95% confidence limits for the three experimentally determined values, were (3.61–3.93) × 10^5 and (1.09–1.33) × 10^5, respectively.) If the partial specific volume of the receptor proteins is taken to be 0.71–0.73 $cm^3\ g^{-1}$, δ_D for the two forms identified here is 0.37–0.53 g per g of protein. The molecular weights of the protein parts of the complexes ($R1_p$ and $R2_p$) are then (2.46–2.75) × 10^5 and (0.79–0.88) × 10^5, respectively, and the complexes contain 160–200 and 50–65 mol of TX100 per mol of protein. The frictional ratios of the complexes are 1.45 and 1.08 for R1 and R2, respectively.

Discussion

Integral membrane proteins usually contain areas of their surface which are very hydrophobic, and their solubilization involves the binding of detergents to these areas, screening them from the aqueous environment (Simons et al., 1973; Spatz & Strittmatter, 1973). Therefore, it is necessary to take into account that one is studying a protein–detergent (and possible protein–lipid–detergent) complex when characterizing solubilized membrane proteins.

The use of a combination of gel filtration and sucrose density gradient centrifugation to determine the molecular weight of a protein in a mixture of proteins was first suggested and applied by Siegel & Monty (1966) and has since been applied to several solubilized membrane proteins (e.g., Neer, 1974; Stengel & Hanoune, 1979; Costrini et al., 1979). When applied to detergent-solubilized proteins, however, the method suffers from the inherent assumption, pointed out by Tanford et al. (1974), that the molecular parameters determined (a, $s_{20,w}$, and $\bar{v}$) remain constant in the different media employed. The major source of change is likely to be alterations in detergent binding; high concentrations of sucrose or high ionic strength media alter the strength of hydrophobic interactions (Hjelmeland et al., 1978). Sucrose also alters the degree of hydration of molecules in the same solution, thereby altering their frictional coefficient, diffusion coefficient, and bouyant density. Changes in aggregation state, as observed for isolated R1 and R2 in sucrose gradients, may indicate a change in the amount of detergent bound or a direct effect on the binding between protein moieties. Sucrose appears to alter the aggregation state of Semiliki Forest virus membrane protein solubilized in TX100 (Simons et al., 1973) and of tubulin, a water-soluble protein (Frigon & Lee, 1972). We found, however, that gel filtration of photoprobe-labeled receptor in 10% sucrose or 2 M NaCl had no significant effect on the Stokes radius of R1 or R2, which confirms the findings of Cuatrecasas (1972) for the active binding receptor in TX100 solutions.

While it is probable, therefore, that the hydration of, and/or detergent binding to, the receptor proteins are not invariant in the different media used, the changes in the hydrodynamic parameters determined appear to be less than the 10% error expected for gel filtration (Nozaki et al., 1976).

Studies with the TX100-solubilized insulin receptor (Cuatrecasas, 1972; Ginsberg et al., 1976; Maturo & Hollenberg, 1978; Sahyoun et al., 1978) have found values of Stokes' radii for insulin-binding components of the receptor of 7.0–7.2 and 3.8–4.0 nm. The difference between these and our value for the smaller form is probably due to our more extended range of calibrating proteins. Cuatrecasas also measured the $s^{0.734}_{20,w}$ of the insulin receptor; the difference between his value (11.0 S) and ours (10.15 S) appears to be due to differences in values of $s_{20,w}$ for calibrating proteins used [cf. Figure 4 of Cuatrecasas (1972)].

The interpretation of frictional ratios of TX100–protein complexes is made uncertain by the high degree of hydration of the poly(oxyethylene) chains that form the hydrophilic section of the Triton molecule (Greenwald & Brown, 1954). However, the high value of f/f_0 for R1 indicates a considerable degree of asymmetry, which would explain the very high value for the molecular weight of the receptor determined by Lang et al. (1980b) by comparing the receptor with globular proteins.

The estimated values for the molecular weight of the smaller form of the receptor are in reasonable agreement with the molecular weight determined by Ferguson plot (9.0 × 10^4; Wisher et al., 1980) and by radiation inactivation (8.7 × 10^4; Harmon et al., 1980). This suggests that the small form is the single polypeptide identified as the insulin-binding component of the receptor.

The unusually low value of f/f_0 for R2 is of interest, as it suggests a globular shape for the complex. It is unlikely that a nearly globular protein of this size would span the membrane and implies that the insulin-binding component is located in the exterior surface of the membrane only.

We have found that the dissociation of the insulin binding component from the receptor and its reaggregation with other components, which appear to be of similar size and hydrodynamic density in TX100 solution, are possible in the absence of chemical modification. The dissociation of the TX100-solubilized receptor has also been induced by high concentrations of insulin (Ginsburg et al., 1976; Krupp & Livingston, 1978) and by affinity purification of the receptor on insulin–agarose (Maturo & Hollenberg, 1978). These findings are in agreement with our own findings that the 3.6-nm form of the receptor is only observed by gel filtration after the covalent attachment of an insulin analogue and not by measuring binding activity in unlabeled solubilized membranes. In preliminary experiments in which D_2O was used as the solvent for sucrose density gradients, thereby altering the surface properties of the gradient medium, the proportion of R1 was increased from 40% to 55% relative to the same material analyzed on H_2O–sucrose gradients. This result, together with the observed increase in dissociation in sucrose, suggests that the insulin-binding component is bound to other components of the receptor by hydrophobic interactions alone.

There have been two reports (Pilch & Czech, 1980; Jacobs et al., 1980) that the dissociation of the affinity-labeled receptor into subunits requires reduction of interchain disulfide bonds. These studies are, however, based on the comparison of the migration of the reduced and nonreduced receptor in $NaDodSO_4$–polyacrylamide gel electrophoresis. The determination of molecular weight by $NaDodSO_4$–polyacrylamide gel electrophoresis requires the reduction of disulfide bonds in order to ensure complete unfolding of the polypeptides and uniform binding of $NaDodSO_4$ (Griffith, 1972). We have found that in the absence of disulfide reduction, photoaffinity-labeled receptor is extremely resistant to solubilization by $NaDodSO_4$. Less than 50% of the radioactivity solubilized in the presence of β-mercaptoethanol is solubilized in its absence. Water-soluble protein markers that behaved normally on $NaDodSO_4$–polyacrylamide gel electrophoresis after reduction showed decreased mobility if not reduced. This decrease was most noticeable with higher molecular weight polypeptides, with the result that the usual (log molecular weight) vs. (relative mobility) plot was curvilinear over its entire range. The interpretation of migration of proteins with intact disulfide bonds on $NaDodSO_4$–polyacrylamide gel electrophoresis should therefore be treated with caution.

We have found, however, that reduction of disulfide bonds greatly promotes the dissociation of the IBC from the other

receptor components. Given that dissociation can occur in the absence of chemical modification, this indicates that intrachain cysteines in one or more components of the receptor are necessary to maintain the conformation required for the association of the different components of the active receptor.

Acknowledgments

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