Effects of Octyl β-Glucoside on Insulin Binding to Solubilized Membrane Receptors†

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ABSTRACT: Octyl β-glucoside (1%), a dialyzable detergent, was used to solubilize the insulin receptor of the turkey erythrocyte membrane. Insulin binding capacity was stable for at least 1 week when the receptor was kept in 1% octyl β-glucoside at 4 °C. The binding properties of the solubilized receptor were examined at detergent concentrations above (1%) and below (0.6%) the critical micelle concentration. A reduction in insulin binding occurred when the detergent concentration was raised above the critical micelle concentration, due to an apparent decrease in the number of binding sites. The specificity of the receptor for insulin analogues was preserved, and the relative affinity of the solubilized receptor, desoctapeptide insulin < proinsulin < porcine insulin, was similar in 0.6% and 1% detergent. Addition of divalent cations increased insulin binding to a similar extent at both detergent concentrations, but there was a slightly greater stimulation of binding in 0.6% detergent as compared to 1% detergent. The pH optimum for binding was not affected by changes in the detergent concentration. These results indicate that the insulin receptor can be successfully solubilized by octyl β-glucoside and that the binding activity is quite stable. Therefore, octyl β-glucoside may be a useful detergent for purification of this receptor. In addition, the data indicate that the binding properties of the insulin receptor can be affected by changes in the physical state of the octyl β-glucoside.

The insulin receptor has been solubilized from a number of sources, including turkey erythrocyte membranes, by using Triton X-100 (Ginsberg et al., 1978; Maturo & Hollenberg, 1978; Krupp & Livingston, 1978). Although the properties of the insulin receptor are not altered appreciably by Triton X-100 (Ginsberg et al., 1978), several undesirable effects of the detergent led us to explore the feasibility of using octyl β-glucoside in its place. On is that Triton X-100 is extremely difficult to remove except by treatment with Bio-Beads SM-2 (Holloway, 1973)? We have found that this procedure results in significant protein and lipid loss from solubilized receptor preparations. In addition, Triton X-100 cannot be easily removed during reconstitution procedures. Finally, attempts to purify the insulin receptor from Triton X-100 extracts have resulted in low yields, and the preparations have unstable binding activity (Ginsberg et al., 1978; Harrison & Itin, 1980). Our preliminary studies indicated that the insulin receptor could be solubilized by octyl β-glucoside and that the use of this detergent overcame several of the problems associated with Triton X-100 (Gould et al., 1979).

Octyl β-glucoside, a nonionic detergent, is an effective agent for solubilizing membrane proteins (Baron & Thompson, 1975; Stubbs & Litman, 1978a,b; Petri & Wagner, 1979; Schneider et al., 1980; Gould et al., 1979), and it has been employed recently to solubilize receptors from membranes in a form suitable for purification (Petri & Wagner, 1979; Schneider et al., 1980). However, it is reported to affect the properties of some membrane proteins; for example, it alters the state of association of cytochrome *c* oxidase and decreases the activation free energy for the loss of opsin regenerability by displacing phospholipids (Rosevear et al., 1980; Stubbs & Litman, 1978b). In spite of these deleterious effects, the high critical micelle concentration facilitates reconstitution of the insulin receptor from this detergent (Gould et al., 1979). Because of its potential usefulness for insulin receptor studies, we wished to examine systematically the affects of this detergent on the binding characteristics of the solubilized insulin receptor.

Materials and Methods

Isolation of Membranes. Turkey erythrocyte membranes were prepared as described earlier with minor modifications (Ginsberg et al., 1976; Gould et al., 1979). Turkey blood was collected from a local abattoir with 1 mg/mL heparin as the anticoagulant. The blood cells were sedimented at 1700*g* for 5 min at 4 °C. After the plasma and buffy coat were removed by aspiration, the erythrocytes were resuspended into 150 mM NaCl and resedimented at 1700*g*. This washing procedure was done 3 times, and the erythrocytes were then stored overnight at 4 °C in 150 mM NaCl. Hemoglobin was removed by repeated cycles of hypotonic lysis in 8.5 mM Tris-HCl, pH 7.8, 3 mM NaCl, 1 mM glucose, and 2 mM $MgCl_2$, followed by resedimentation at 4000*g* for 10 min at 4 °C. After all of the hemoglobin was removed as indicated by the presence of a colorless to light pink supernatant fluid, a final wash was performed with the buffered salt solution containing 0.2 mM $MgCl_2$ instead of 2 mM $MgCl_2$. Nuclei were extruded from the membranes by Dounce homogenization, the extent of extrusion being monitored by phase-contrast microscopy. Typically, 15–20 strokes with a tight-fitting pestle were required to give 70–80% separation of nuclei and membranes. The nuclei were then removed from the membranes by centrifugation at 5000*g* for 10 min. A pellet containing two layers formed, with the less dense membranes segreting in the upper, lighter colored layer. This membrane fraction was removed by using a syringe fitted with a 16-gauge needle, and the membranes were washed until no highly refractory nuclei were visible by phase-contrast microscopy. The amount of DNA contamination and the specific activity of the ouabain-sensitive Na^+/K^+-ATPase were monitored as measures of membrane purity. DNA was measured by reaction with the diphenylamine reagent (Keleti & Lederer, 1974). Ouabain-sensitive Na^+/K^+-ATPase activity was determined as previously described (Solomonson et al., 1976). Inorganic phosphate release was determined by a modification of the method of Fiske & Subbarow (1925). In this assay, protein was removed by precipitation with 10% (w/v) trichloroacetic acid. Subse-

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quently, 1 mL of the supernatant solution was added to 1 mL of 1.25% ammonium molybdate in 2.5 N H_2SO_4 and 1 mL of 1% Elon (Eastman Kodak Chemicals) in 3% sodium bisulfite. After incubation for 15 min at room temperature, the absorbance was determined at 660 nm. Membranes typically hydrolyzed 0.97–1.1 μmol of P_i (mg of protein)$^{-1}$ h^{-1} from ATP and contained less than 10 μg of DNA/mg of protein. Protein concentration was determined by the method of Lowry as modified by Peterson (1977).

Extraction of Soluble Binding Material. Unless otherwise indicated, membranes were extracted with a final concentration of 1% (w/v) octyl β-glucoside in a buffer solution containing 85 mM Tris-HCl, pH 7.8, 30 mM NaCl, 10 mM glucose, and 1 mM EDTA (buffer I). Extractions were carried out at 2.9 mg/mL membrane protein for 15 min at room temperature. Insoluble material was removed by sedimentation at 104000*g* for 1 h at 4 °C, and the supernatant solution containing the solubilized membrane proteins was collected. The insulin binding activity contained in this solubilized material was stable at −20 °C for at least 1 month and at 4 °C for at least 1 week. Membrane proteins also were solubilized with 1% Triton X-100 as has been described (Ginsberg et al., 1978).

Binding Analysis. [^{125}I]Iodoinsulin was prepared by a modification of the chloramine T method (Ginsberg et al., 1976). The specific activity of the [^{125}I]iodoinsulin preparation varied from 150–200 Ci/g. Insulin binding activity was measured in 0.5 mL of buffer I containing 1 mg/mL bovine serum albumin and approximately 20 pM [^{125}I]iodoinsulin. Octyl β-glucoside was added to give a final concentration of either 0.6% or 1%. Nonspecific or nonsaturable binding was defined as the [^{125}I]iodoinsulin which bound in the presence of 2 μg/mL porcine insulin. Bound insulin was separated from unbound insulin by precipitating the receptor–insulin complex with 0.5 mL of 25% poly(ethylene glycol) plus 0.1 mL of bovine γ-globulins (5 mg/mL). After the mixture was allowed to stand for 5–10 min at room temperature, the precipitate was collected on 0.45-μm cellulose acetate filters (Schleicher & Schuell, Inc.) by using a Bio-Rad filter manifold under a vacuum pressure of 25 mmHg. Radioactivity retained by the filters was determined with a Beckman Gamma 8000 scintillation counter at a counting efficiency of 75%.

Binding data were obtained by incubating the solubilized receptor with tracer amounts of [^{125}I]iodoinsulin plus increasing amounts of unlabeled porcine insulin. The data were plotted by the method of Scatchard, and the binding parameters were calculated by a computerized analysis that fits the competition curve to a fourth order polynomial (B. H. Ginsberg, unpublished results). The program generates a Scatchard plot of the fitted curve and analyzes the data by the models of both negative cooperativity (De Meyts & Roth, 1975) and independent classes of sites (Thakur & Rodbard, 1979). The data are visually presented on a Tectronix 4006 graphics terminal. Porcine insulin was obtained from Elanco, proinsulin was a gift of Dr. Ron Chana, Eli Lilly, and desoctapeptide insulin was a gift from Dr. Joseph Walder, University of Iowa. Insulin degradation was estimated by measuring the amount of [^{125}I]iodoinsulin that was precipitated by 5% trichloroacetic acid after incubation with solubilized membrane protein (Freychet et al., 1972).

Critical Micelle Concentration Determination. The micelle formation of octyl β-glucoside in buffer I was determined by using the fluorescent probe *N*-phenyl-1-naphthylamine (Aldrich Chemical Co). (Williams et al., 1979). Fluorescence measurements were made with a Hitachi Perkin-Elmer MPF-2A fluorescence spectrophotometer. Excitation was performed at 360 nm, and the fluorescent emission was measured at 425 nm. Micelle formation was determined by the increase in quantum yield of *N*-phenyl-1-naphthylamine in a hydrophobic environment of a micelle (Radda, 1971). Fresh reagent was prepared immediately before use, containing 34.3 mg of *N*-phenyl-1-naphthylamine dissolved in 25 mL of methanol. For the assay, 2 μL of this stock solution was added to 0.5 mL of detergent solution, giving a final *N*-phenyl-1-naphthylamine concentration of 25 μM. The fluorescence of *N*-phenyl-1-naphthylamine was maximal when bovine serum albumin was included in buffer I, whether or not octyl β-glucoside was present. Therefore, it was necessary to omit albumin from the solution when the critical micelle concentration was measured by this technique. In addition, micelle formation in the presence and absence of 1 mg/mL bovine serum albumin in buffer I was monitored by measuring the light scattering at 250 nm as the octyl β-glucoside concentration was raised.

Circular Dichroism Studies. Circular dichroism spectra of insulin were obtained with a Cary 60 spectrophotometer in the CD mode. Spectra were obtained at octyl β-glucoside concentrations of either 0.6% of 1% in buffer I, containing either 55 or 110 μM insulin. Path lengths of 0.1 and 0.05 cm were employed, respectively. Insulin concentrations were determined spectrophotometrically after circular dichroism measurements, assuming a molar extinction coefficient of 0.57 × 10^{-4} at 280 nm and a molecular weight of 5734. Mean-residue-weight ellipticities were calculated at 208 and 222 nm, assuming a mean residue molecular weight of 112 (Goldman & Carpenter, 1974). Spectra were obtained at room temperature, approximately 20 °C, with a full-scale deflection of 0.1°.

Results

Solubilization of Receptors and Protein Dependence of Binding. Turkey erythrocyte membranes (2.9 mg/mL) were incubated with 1% octyl β-glucoside at 20 °C. After 15 min, 7.3% of the membrane protein was solubilized. Incubations with higher concentrations of octyl β-glucoside resulted in greater amounts of protein being solubilized, but no further increase in the amount of insulin binding activity was solubilized. Thus, the apparent specific activity of the soluble insulin receptor actually was less at the higher detergent concentrations. Therefore, 15 min at 20 °C in 1% octyl β-glucoside was selected for all subsequent solubilization studies.

Since Triton X-100 has been employed previously to solubilize the insulin receptor from turkey erythrocyte membranes (Ginsberg et al., 1978), we compared directly octyl β-glucoside and Triton X-100. Figure 1 demonstrates the protein dependence of [^{125}I]iodoinsulin binding to solubilized membrane material, assayed in either 1% octyl β-glucoside or 0.05% Triton X-100. At comparable protein concentrations, [^{125}I]-iodoinsulin binding was much greater with material solubilized in octyl β-glucoside than in Triton X-100. The nonpsecific binding or, more correctly, the nonsaturable binding had a bound/free ratio of less than 0.002, even at the highest protein concentrations tested. All data have been corrected for this low amount of nonspecific binding.

After incubation of 15 pM or 0.3 μM [^{125}I]iodoinsulin with the soluble receptor for 4 h at 15 °C, 99.2 ± 0.9% ($\bar{x}$ ± SE; n = 4) of the radioactivity at 15 pM or 101.5 ± 1.8% ($\bar{x}$ ± SE; n = 4) at 0.3 μM was precipitated by trichloroacetic acid. Since the major degradation products of insulin are soluble in trichloroacetatic acid (Caro & Amatruda, 1980), the majority of the [^{125}I]iodoinsulin was not degraded during the binding assay.

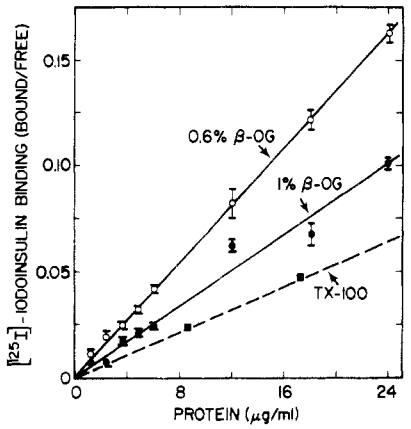

FIGURE 1: Protein dependence of insulin binding. Membrane protein was solubilized with 1% octyl β-glucoside (solid line) or 1% Triton X-100. These preparations were incubated with 21 pM [^{125}I]iodoinsulin and either 0.6% (○) or 1% (●) octyl β-glucoside or 0.05% Triton X-100 (■). Incubations were performed for 4 h at 15 °C and terminated by addition of poly(ethylene glycol). Binding has been corrected for nonspecific binding as determined in the presence of 2 μg/mL insulin. Each data point is the average of three separate determinations. Error bars indicate the standard deviation.

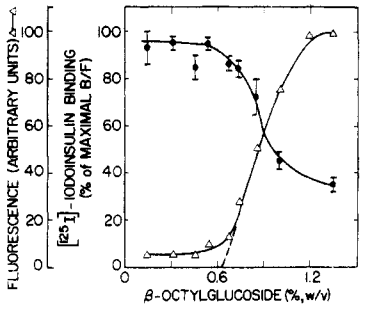

FIGURE 2: Effect of octyl β-glucoside concentration on insulin binding. Solubilized membrane protein (10 μg) was incubated with 20 pM [^{125}I]iodoinsulin and increasing amounts of octyl β-glucoside. Incubations were carried out for 4 h at 15 °C and terminated by addition of poly(ethylene glycol). Binding (●) has been corrected for nonspecific binding as determined in the presence of 2 μg/mL insulin. Relative fluorescence of N-phenyl-1-naphthylamine (△) was determined in buffer I and increasing amounts of octyl β-glucoside. Each data point is the average of four separate determinations. Error bars indicate the standard deviation.

Effect of Octyl β-Glucoside Concentration on Binding. Figure 2 demonstrates the effect of octyl β-glucoside concentration on insulin binding to the solubilized membrane protein preparation. A 65% decrease in binding of [^{125}I]-iodoinsulin occurred as the detergent concentration was raised from 0.15 to 1.2%, although the greatest loss in binding occurred between 0.6% and 1%. This reduction was associated with the formation of detergent micelles, as indicated by the increase in fluorescence of *N*-phenyl-1-naphthlamine. The critical micelle concentration under these conditions was determined to be 0.66% by extrapolation of the fluorescence data (dashed line). A critical micelle concentration of 0.64% in the absence of albumin and of 0.65% in the presence of 1 mg/mL albumin was determined by measuring light scattering at 250 nm. This verifies the results obtained by the fluorescent probe and indicates that the presence of albumin has no effect on the micelle formation of octyl β-glucoside. Therefore, no micelle formation occurred when the detergent concentration was less than 0.6%. Figure 2 also shows that there was no significant decrease in insulin binding when the octyl β-glucoside concentration was lowered under conditions where micelles did not form.

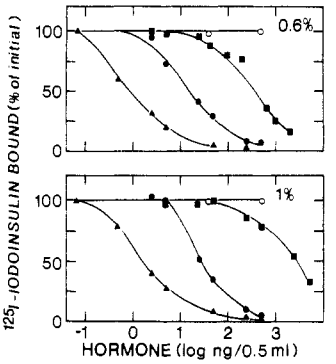

FIGURE 3: Comparative relative binding affinities. Solubilized membrane protein (15 μg) was incubated with 23 pM [^{125}I]iodoinsulin and either 0.6% (top panel) or 1% (bottom panel) octyl β-glucoside. Unlabeled porcine insulin (▲), proinsulin (●), desoctapeptide insulin (■), or human growth hormone (○) was added to give the indicated final concentration. Incubations were carried out for 4 h at 15 °C and terminated by addition of poly(ethylene glycol). Binding has been corrected for nonspecific binding as determined in the presence of 2 μg/mL insulin. Each data point is the mean of three separate determinations.

We then compared the binding properties of the receptor in either 0.6% or 1% octyl β-glucoside. Figure 1 demonstrates the protein dependence of [^{125}I]iodoinsulin binding to solubilized membrane material. Specific binding increased linearly with increasing protein in both detergent concentrations. [^{125}I]Iodoinsulin binding was depressed at all protein concentrations when the assay was carried out in 1% octyl β-glucoside as compared to 0.6% octyl β-glucoside.

Since octyl β-glucoside affected insulin binding, we wished to determine whether this may be due to interactions of the detergent with insulin. Circular dichroism spectra at 55 and 110 μM insulin provided no evidence, however, that 1% or 0.6% octyl β-glucoside appreciably altered the insulin structure.

Binding Properties of Solubilized Receptor in 0.6% and 1% Octyl β-Glucoside. The insulin receptor retained its specificity for insulin after solubilization with octyl β-glucoside. Figure 3 shows the competition for binding of [^{125}I]iodoinsulin to the solubilized insulin receptor by procine insulin, proinsulin, and desoctapeptide insulin. The binding of [^{125}I]iodoinsulin was reduced to 50% of maximal values by porcine insulin at 1.6 ng/mL in 0.6% octyl β-glucoside and 3.2 ng/mL in 1% octyl β-glucoside, by proinsulin at 40 ng/mL in 0.6 octyl β-glucoside and 63 ng/mL in 1% β-octyl β-glucoside, and by desoctapeptide insulin at 800 ng/mL in 0.6 octyl β-glucoside and 5000 ng/mL in 1% octyl β-glucoside. Human growth hormone, 1 μg/mL, did not displace [^{125}I]iodoinsulin from the receptor. The relative molar potencies of insulin, proinsulin, and desoctapeptide insulin in displacing [^{125}I]iodoinsulin were calculated to be 1:0.078:0.001 in 1% detergent and 1:0.063:0.003 in 0.6% detergent. Although these values are decreased somewhat as compared to those of the insulin receptor contained in the intact turkey erythrocyte membrane (Ginsberg, et al., 1976), they are similar to the biological activities measured for these analogues by glucose oxidation (Freychet et al., 1971) and demonstrate that the specificity of the insulin receptor is maintained when it is solubilized by octyl β-glucoside.

A characteristic feature of insulin binding to its receptor is the inverse relationship between steady-state binding levels and temperature (Ginsberg et al., 1976; Ginsberg, 1977). This characteristic was not affected by solubilization with octyl β-glucoside, for the equilibrium level of binding at 37 °C was less than that at 15 °C, which in turn was less than that at 0 °C when assayed in either 0.6% or 1% octyl β-glucoside.

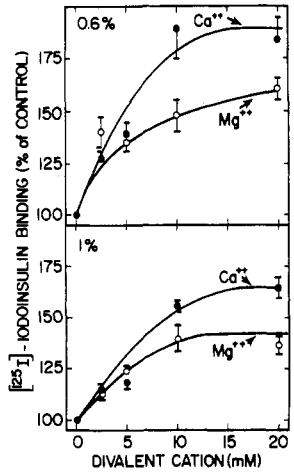

FIGURE 4: Effect of divalent cations on insulin binding. Ten micrograms of solubilized membrane protein was incubated with 20 pM [^{125}I]iodoinsulin and 0.6% octyl β-glucoside (top panel) or 15 μg of solubilized membrane protein, 20 pM [^{125}I]iodoinsulin, and 1% octyl β-glucoside (bottom panel). Ca^{2+} (●) or Mg^{2+} (○) was added as the chloride salt. Incubations were carried out for 4 h at 15 °C and terminated by addition of poly(ethylene glycol). Binding has been corrected for nonspecific binding as determined in the presence of 2 μg/mL insulin. Each data point is the average of three separate incubations.

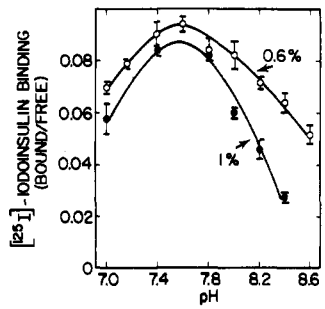

FIGURE 5: pH optimum for binding of insulin to the solubilized membrane receptor. The pH optimum was determined in either 0.6% (○) or 1% (●) octyl β-glucoside and a buffer solution containing 50 mM Tris, 50 mM Hepes, 60 mM NaCl, 10 mM glucose, and 1 mg/mL bovine serum albumin, adjusted to the appropriate pH at 15 °C. Nonspecific binding, which has been subtracted from each data point, showed no pH dependence. Each data point is the mean of three separate determinations.

Another unique feature of insulin binding to the receptor of the avian erythrocyte is the dependency on divalent cations (Ginsberg et al., 1977). As shown in Figure 4, addition of Mg^{2+} and Ca^{2+} increased the binding similarly when the receptor was contained in either 0.6% or 1% octyl β-glucoside. In 0.6% octyl β-glucoside, 20 mM Ca^{2+} increased the binding to 185% of the level observed in the absence of Ca^{2+}, while in 1% octyl β-glucoside the increase was to 164%. Likewise, 20 mM Mg^{2+} increased the binding to 162% in 0.6% octyl β-glucoside and 138% in 1% octyl β-glucoside. Calcium consistently increased the binding more than magnesium. These results indicate that the insulin receptor solubilized in octyl β-glucoside responds similarly with respect to divalent cations as the receptor solubilized in Triton X-100 and the receptors contained in the intact turkey erythrocyte (Ginsberg et al., 1978, 1977). On the other hand, the insulin receptor solubilized with either detergent is more responsive to increasing divalent cation concentrations than the receptor present in the isolated turkey erythrocyte membrane (Ginsberg et al., 1976, 1978).

Figure 5 demonstrates that the pH optimum of insulin binding to the solubilized receptor is not affected by the octyl β-glucoside concentration. Maximal binding was observed between pH 7.4 and 7.8. The reduction in binding at higher pH values, however, was much less pronounced when the receptor was present in 0.6% than in 1% octyl β-glucoside. This pH optimum is slightly higher than that observed in Triton X-100 but lower than that for the receptor contained in the turkey erythrocyte membrane (Ginsberg et al., 1976, 1978).

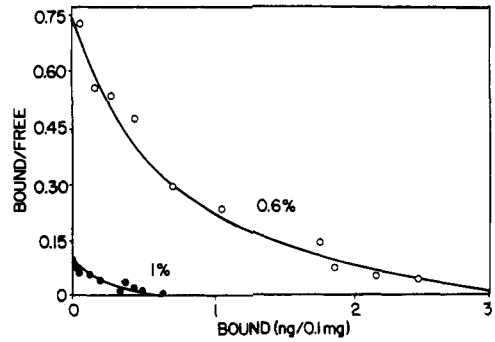

FIGURE 6: Scatchard analysis of binding data. Solubilized membrane protein (10 μg) was incubated with 20 pM [^{125}I]iodoinsulin and either 0.6% (○) or 1% (●) octyl β-glucoside. Unlabeled insulin was added to vary the final concentration of insulin from 20 pM to 0.18 μM. Incubations were carried out for 4 h at 15 °C and terminated by addition of poly(ethylene glycol). Binding has been corrected for nonspecific binding as determined in the presence of 2 μg/mL insulin. The binding data were analyzed by using a computer program. Results are shown from a representative experiment.

Table I: Parameters from Scatchard Analysis of Binding Data[a]

Model I: Two Nonidentical Classes of Sites				
octyl β-glucoside concn (%)	K_1 (nM^{-1})	K_2 (nM^{-1})	n_1 (pmol/mg)	n_2 (pmol/mg)
0.6	1.47 ± 0.40	0.24 ± 0.12	1.16 ± 0.05	3.08 ± 1.15
1	1.50 ± 0.10	0.14 ± 0.04	0.56 ± 0.32	1.32 ± 0.22

Model II: Negative Cooperativity			
octyl β-glucoside concn (%)	$\bar{K}_e$ (nM^{-1})	$\bar{K}_f$ (nM^{-1})	n (pmol/mg)
0.6	0.64 ± 0.11	0.22 ± 0.04	4.55 ± 0.72
1	0.51 ± 0.06	0.17 ± 0.08	1.6 ± 0.76

[a] $\bar{x}$ ± SE of three (1%) or four (0.6%) separate determinations.

Binding Analysis. In order to determine whether the binding decrease produced by the detergent was due to a reduced affinity of the receptor or a reduction in the number of available binding sites, we performed Scatchard analyses of the binding data obtained in 0.6% and 1% octyl β-glucoside. Figure 6 demonstrates the Scatchard plots obtained, and Table I summarizes the binding parameters calculated from an analysis of these Scatchard plots. The binding models employed assume either negative cooperativity among insulin receptors or the presence of two nonidentical classes of receptors. A similar result was obtained when either of these binding models were employed. Both analyses indicated that as the detergent concentration is raised, there is a loss of available insulin binding sites but little change in the affinity of the receptor.

Discussion

We have demonstrated that the properties of the insulin receptors in octyl β-glucoside are remarkably similar to those observed in other nonionic detergents and intact membranes (Ginsberg et al., 1978; Gavin et al., 172; Ginsberg, 1977). Because of the observed stability of the insulin receptor in octyl β-glucoside and the ability to remove the detergent by gel

filtration chromatography and dialysis, the use of this detergent may permit greater ease in receptor purification with retention of binding activity. This has proved to be extremely difficult with existing procedures that utilize Triton X-100 (Harrison & Itin, 1980; Ginsberg et al., 1978). Recently, the insulin binding activity of human placenta has been solubilized successfully with octyl β-glucoside (R. Noble and B. Ginsberg, unpublished experiments). Therefore, this detergent may have general applicability for insulin receptor solubilization and binding studies.

Insulin binding data obtained with the receptor solubilized in either 0.6% or 1% octyl β-glucoside were curvilinear when plotted according to the method of Scatchard. The curvilinear plot is a characteristic feature of the insulin receptor and has been attributed to negative cooperativity between homogeneous sites (De Meyts et al., 1973, 1976), the presence of two noninteracting classes of sites (Pollet et al., 1977; Caro & Amatruda, 1980), or the rebinding of insulin fragments following degradation of intact insulin (Donner, 1980). Since [^{125}I]-iodoinsulin incubated with the solubilized receptor was fully precipitated by trichloroacetic acid, it is unlikely that the curvilinear Scatchard plot in this case is due to rebinding of insulin fragments. Because of the controversy regarding negative cooperativity as opposed to independent classes of sites (Pollet et al., 1977, 1980), the first two models were both employed to analyze our binding data. Independent of the model selected, the results indicate that a marked decrease in the number of available insulin binding sites occurred when the amount of octyl β-glucoside was raised above the critical micelle concentration. The molecular mechanism of the decrease or masking of binding sites produced octyl β-glucoside cannot be determined from the present studies. In biologic membranes, perturbation of the lipid components results in a 4–6-fold increase in the amount of insulin binding. This has been ascribed to an increase in the number of available binding sites (Cuatrescasas, 1971; Ginsberg et al., 1979). Therefore, the capacity to mask available binding sites on the insulin receptor apparently is not dependent on the presence of a specific amphipathic molecule, for the presence of either phospholipids contained in the biologic membrane or octyl β-glucoside in the form of detergent micelles produces similar effects. The possibility that alterations in insulin conformation are responsible for the detergent-induced changes in binding seems unlikely because the circular dichroism studies provide no evidence for a pronounced alteration in insulin structure when the octyl β-glucoside concentration was raised from 0.6% to 1%. Since these measurements reflect the overall secondary structure of insulin, it is possible that the detergent produced small conformational changes at specific sites responsible for binding to the receptor but that they were too small to be detected by circular dichroism measurements. Furthermore, in order to obtain circular dichroism spectra, it was necessary to use much higher insulin concentrations than were employed for the binding experiments. It is possible that certain detergent effects which occur at low insulin concentrations are not seen at higher concentrations because of dimer formation. This is another reason why the circular dichroism data do not completely exclude octyl β-glucoside effects on the hormone itself.

The finding that the solubilized insulin receptor responds to alterations in the physical state of octyl β-glucoside may be applicable to other membrane proteins. A number of membrane-bound proteins including cytochrome *c* oxidase (Rosevear et al., 1980), opsin (Stubbs & Litman, 1978b), the low density lipoprotein receptor (Schneider et al., 1980), and the glycoprotein of vesicular stomatitis virus (Petri & Wagner, 1979) have been solubilized by octyl β-glucoside. In light of the present results, it is possible that data obtained with these proteins above and below the detergent critical micelle concentration also may not be directly comparable. Therefore, this possibility should be considered when octyl β-glucoside is utilized for membrane solubilization studies.

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Association of the Thyroid Hormone Receptor with Rat Liver Chromatin†

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ABSTRACT: We have investigated the association of the triiodothyronine (T_3) nuclear receptor with rat liver chromatin by the use of selective endonuclease digestion and differential solubilization. The T_3 receptor was found in a fraction of chromatin having some of the characteristics of active chromatin: (a) It is highly sensitive to DNase I and micrococcal nuclease digestion; (b) it is enriched in nonhistone proteins and depleted of histone 1 (H-1). Micrococcal nuclease and pancreatic DNase I excised two receptor-containing fragments from chromatin, a minor (12–14 S) form and a major (5.5–6.0 S) form. The latter structure has a Stokes radius of 42 ± 2 Å and an estimated molecular weight of 95 400 when a partial specific volume of 0.725 cm^3/g for protein is used. It contains DNA but no histones. Similar receptor-containing fragments were excised from chromatin of other rat tissues, including brain, kidney, and heart. Both the 5.5–6 S and the 12–14 S receptor-containing chromatin structures are converted by 0.5 M KCl to the 3.5 S form (R_0 35 Å molecular weight 50 500). Titration with micrococcal nuclease and pancreatic DNase I revealed that the 5.5–6 S form is preferentially excised by endonucleases. Neither receptor occupancy nor thyroidal status had an apparent effect on the susceptibility of chromatin to endonucleolytic digestion nor did they influence the distribution of T_3 receptors in chromatin. Our results suggest that T_3 receptors are not tightly associated with nucleosomes, the basic subunit of chromatin, but are associated with the DNA-linking nucleosomes in structurally modified regions of chromatin in rat liver nuclei. The T_3 receptor-containing fragment may well represent a higher order of structural complexity necessary for T_3 action at the cellular level.

Recently, three laboratories independently reported that micrococcal nuclease excised a triiodothyronine (T_3)[1] receptor-containing fragment from chromatin as a 5.0–6.5 S complex in GH_1 pituitary cells (Samuels et al., 1980) and rat liver (Jump & Oppenheimer, 1980; Groul, 1980). Our studies indicated (Jump & Oppenheimer, 1980) that T_3 receptors in euthyroid hepatic nuclei are associated with a fraction of chromatin which is highly sensitive to both pancreatic DNase I and micrococcal nuclease digestion and suggested that T_3 receptors were associated with the expressed region of chromatin in euthyroid liver nuclei. These inferences were based on the work of others who had demonstrated the selective digestion of transcriptionally active genes by DNase I (Weintraub & Groudine, 1976; Garel & Axel, 1977) and micrococcal nuclease (Bellard et al., 1978; Bloom & Anderson, 1978; Levy-Wilson et al., 1979).

Because of the importance of receptor occupancy in mediating T_3 effects on nuclear function (Oppenheimer, 1979), we were interested in determining whether thyroidal status or receptor occupancy influences the structure and composition of chromatin, especially those regions which contain the T_3 receptor in rat liver nuclei. In this report, we have used two methods, endonuclease digestion of intact nuclei and differential solubility, to fractionate chromatin. The analysis examines the organization of chromatin neighboring the T_3 receptor binding site, as well as the distribution of T_3 receptors in isolated chromatin fragments. We were also interested in determining whether T_3 receptors in other receptor-containing tissues are also associated with chromatin structures similar to those in rat liver.

Materials and Methods

Animals. Male Sprague-Dawley rats (150–250 g) were used in all experiments. Animals were rendered hypothyroid by surgical thyroidectomy followed by administration of 100 μCi of ^{131}I. Animals were used when no further weight gain was evident, i.e., 4–5 weeks following radioablative treatment. Euthyroid and hypothyroid animals receiving in vivo administered [^{125}I]T_3 (20–50 ng/100 g of body weight, 450–550 μCi/μg; Abbott Laboratories) were injected via the tail vein and killed 30 min later unless otherwise stated. During the period, the metabolism of injected tracer T_3 is negligible, and nuclear radioactivity is in the form of [^{125}I]T_3 (Oppenheimer et al., 1974a). Greater than 95% of the nuclear [^{125}I]T_3 is specifically bound to receptors on the basis of studies in which [^{125}I]T_3 (50 ng/100 g of body weight) is injected with and without 10 μg of unlabeled T_3.

Preparation of Hepatic Nuclei. Rat liver nuclei were prepared as previously described (Oppenheimer et al., 1974b) with minor modifications (Jump & Oppenheimer, 1980). Crude nuclear pellets obtained by centrifugation through 2.4 M sucrose were resuspended in 6 mL of buffer C (0.25 M sucrose, 10 mM Tris, pH 8.0, 2 mM $CaCl_2$, 2 mM $MgCl_2$, 1 mM DTT, and 0.4 mM PMSF) per 3 g equiv of tissue. The nuclear suspension was adjusted to 1% Triton X100, and nuclei

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[1] Abbreviations used: T_3, triiodothyronine; PMSF, phenylmethanesulfonyl fluoride; DTT, dithiothreitol; $NaDodSO_4$, sodium dodecyl sulfate; Cl_3CCOOH, trichloroacetic acid.

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