Hydrodynamic Properties of the \( \beta \)-Adrenergic Receptor and Adenylate Cyclase from Wild Type and Variant S49 Lymphoma Cells*

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Cell membranes from clones of cultured S49 mouse lymphoma cells were incubated with \[^{125}\text{I}]\text{iiodohydroxybenzylpindolol, a specific, high affinity ligand for the } \beta \text{-adrenergic receptor, and guanyl-5'-yl imidodiphosphate, which activates adenylate cyclase irreversibly. Membranes were then solubilized with 0.1% Lubrol PX, and hydrodynamic properties of the ligand-receptor complex and of adenylate cyclase were determined by gel filtration and by centrifugation through gradients of sucrose in H_2O or D_2O. Molecular parameters for the } \beta \text{-adrenergic receptor from wild type cells are: Stokes radius, 6.4 nm; } s_{20,w}, 3.1 S; \text{ partial specific volume, 0.83 ml/g; } M_r, 130,000; \text{ frictional ratio, 1.8. The values for adenylate cyclase are: Stokes radius, 7.1 nm; } s_{20,w}, 7.5 S; \text{ partial specific volume, 0.78 ml/g; } M_r, 270,000; \text{ frictional ratio, 1.6. Essentially identical results were obtained with preparations from a variant clone in which the receptor and adenylate cyclase are permanently uncoupled and for the } \beta \text{-adrenergic receptor in a clone that lacks adenylate cyclase activity. The amounts of detergent that are estimated to be bound to the receptor and adenylate cyclase are 0.8 and 0.2 mg/mg of protein, respectively, assuming that the partial specific volume observed represents the weight average for protein and for Lubrol PX. Values of } M_r \text{ for the protein are then calculated to be 75,000 for the } \beta \text{-adrenergic receptor and 220,000 for adenylate cyclase.}

Hormone-sensitive adenylate cyclase systems are envisioned to consist, minimally, of a catalytic moiety and sites for the binding of two required ligands—the hormone and a regulatory nucleotide (presumably a guanine nucleotide). Hypothetically, at least certain of these sites reside on separate polypeptide chains that interact within the bilayer of the plasma membrane. Indirect evidence (kinetic (2, 3), chemical (4, 5), developmental (6), and genetic (7, 8)) supports the hypothesis that the hormone receptor and adenylate cyclase are independent entities. Since specific ligands are now available for the identification of the } \beta \text{-adrenergic receptor in particulate preparations (9, 10) or in solution (11), direct experimentation and physical characterization of both the receptor and adenylate cyclase from the same membranes are now possible. Limbird and Lefkowitz recently reported the separation of these two activities by gel exclusion chromatography (12). This report extends this observation and provides the first information on the physical properties of the } \beta \text{-adrenergic receptor and the adenylate cyclase with which it interacts.}

Previous information on the physical properties of adenylate cyclase comes primarily from the work of Neer (13). She determined that the detergent-solubilized enzyme from rat renal medulla has a partial specific volume similar to that for soluble proteins—consistent with the interpretation that the protein binds little detergent and hence has a minimal surface area devoted to hydrophobic interactions with membrane components. If direct interaction between hormone receptor and enzyme is to occur, it was predicted that the receptor would have a greater hydrophobic surface since it presumably must bind hormone at the external face of the membrane and extend through the bilayer to interact with the catalytic component. The experiments described herein are consistent with this possibility, but they also indicate that the adenylate cyclase of the S49 lymphoma cell may have sufficient hydrophobic surface area to bind significant quantities of detergent.

EXPERIMENTAL PROCEDURES

Cells and Cell Membrane Preparations—The catecholamine-sensitive, wild type clone (24.3.2) of the murine S49 lymphoma cell line was grown to a density of 2 x 10^6/ml in 8-liter spinner cultures of Dulbecco's modified Eagle's medium containing 10% heat-inactivated horse serum. Two variant clones of the S49 lymphoma were also studied: one that is deficient in adenylate cyclase activity (AC-) (14) and another that contains both } \beta \text{-adrenergic receptor and adenylate cyclase but in which ligand binding to the receptor and activation of the enzyme are permanently uncoupled (UNC) (8). Relatively purified membrane fractions were prepared from these clones as described previously (15), and preparations were frozen at -85° until use.

Preparation of Soluble } \beta \text{-Adrenergic Receptor-Ligand Complex and Adenylate Cyclase—Membranes (about 0.2 mg of protein/ml) were incubated with the } \beta \text{-adrenergic receptor-specific ligand, }[^{125}\text{I}]} \text{iiodohydroxybenzylpindolol (50 to 80 pm)} {16), in the presence of Gpp(NH)p (50 } \mu \text{M} \text{ for 30 min at 30°}. This is a sufficient time for

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* The abbreviations used are: Gpp(NH)p, guanyl-5'-y1 imidodiphosphate; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; IHYP, iiodohydroxybenzylpindolol (t-iodo-3-indoloy-1-(2-p-hydroxybenzylpropyl-2-aminoisopropanol).
equilibrium binding of the ligand and for irreversible activation of adenylate cyclase by the guanine nucleotide analog (15). The reaction mixture also contained sodium/Hepes (20 mM, pH 8), MgCl₂ (2 mM), and EDTA (1 mM). Most free radioactive ligand was then removed by filtration for 20 min or by passing the reaction mixture through a column of Sephadex G-50 and charcoal. Membranes were then suspended in one-half the original incubation volume of Lubrol solution (Lubrol PX, 0.1%; sodium/Hepes, 20 mM, pH 8; MgCl₂, 2 mM; EDTA, 1 mM; NaCl, 150 mM), and this suspension was maintained at 4° for 30 to 60 min prior to centrifugation at 24,000 × g for 20 min. Approximately 50% of the adenylate cyclase activity and 80% of the bound [¹²⁵I]iodohydroxybenzylpindolol in the original incubation mixture could be assayed in the total suspension in Lubrol solution; of these, 70% to 90% of each activity was recoverable at this point. This supernatant was then concentrated 4- to 8-fold by ultrafiltration (Diaflo PM30 membrane, Amicon Corp.) and, after addition of calibrating enzymes, was applied to columns to sucrose density gradients.

**Sucrose Density Gradient Centrifugation** - Linear gradients (5 ml) were prepared from 5% and 20% sucrose in H₂O or D₂O containing 7% of volume of 10-fold concentrated Lubrol solution. The densities at 0° were as follows: 5% sucrose in H₂O, 1.030; 20% sucrose in H₂O, 1.089; 5% sucrose in D₂O, 1.123; 20% sucrose in D₂O, 1.171. Samples (0.25 ml) were applied to the tops of gradients and then centrifuged at 4° in the Beckman SW 50.1 rotor at speeds of various times (40,000 to 49,000 rpm, 10 to 15 h). Significant differences in physical parameters were not found over this range of conditions. After centrifugation, the bottoms of tubes were punctured and 20 to 23 fractions of 15 drops each were collected. Column effluent (Ultrogel) was composed of 34.4% ammonium sulfate and 3% acrylamide (0.9 × 27 cm) was eluted and equilibrated with Lubrol solution at 4°. The sample was applied in 0.5 ml and fractions of 0.45 ml were collected. The flow rate was 3.6 ml/h.

**Adenylate Cyclase Assay** - Enzyme activity was measured in a final volume of 100 µl containing [α-³²P]ATP (0.5 mM, 10,000 counts/mg/moL), MgCl₂ (10 mM), NaEDTA (1 mM), 1-methyl-3-isobutylxanthine (0.1 mM), K₂HPO₄ (5 mM), pyruvate kinase (8 µg/ml), bovine serum albumin (0.1 mg/ml), Gpp(NH)p (50 µg/ml), and sodium/Hepes (50 mM, pH 8). Fractions from columns or sucrose gradients (50 µl) were assayed for 30 min at 30°, and the product was isolated by the method of Salomon et al. (17).

**β-Adrenergic Receptor Assay** - The soluble complex of [¹²⁵I]iodohydroxybenzylpindolol and the β-adrenergic receptor was assayed with a polyethylene glycol precipitation technique (18). Fractions from columns or gradients (100 to 200 µl) were mixed with 60 µl of a solution of β-globulin (1%), (±)-propranolol (2 mM), potassium phosphate buffer (20 mM, pH 8), and McIlvaine (1 mM). A solution of polyethylene glycol 6000 (10%), potassium phosphate buffer (20 mM, pH 8), MgCl₂ (5 mM), and KCl (100 mM) was then added (1.2 ml). After incubation at 0° for 20 min, the suspension was filtered (Millipore HAWP, 25-µm diameter); the filter was washed with 5 ml of the polyethylene glycol containing solution at room temperature and counted in a γ counter.

**Calibrating Enzymes** - Calibrating enzymes were added to the solubilized samples at the following concentrations: β-galactosidase (Escherichia coli), 25 µg/ml; fumarase (pig heart), 50 µg/ml; catalase (beef liver), 100 µg/ml; lactate dehydrogenase (rabbit muscle), 30 µg/ml; malate dehydrogenase (pig heart), 10 µg/ml; and cytochrome c (horse heart), 2 mg/ml. The calibrating enzymes were assayed spectrophotometrically under conditions where changes in optical density were proportional to the quantity of protein. The following procedures were used: β-galactosidase at 420 nm with 1 mM o-nitrophenyl-β-D-galactopyranoside (19), fumarase at 240 nm with 50 mM L-malate (20), catalase at 240 nm with 0.006 % H₂O₂ (20), lactate dehydrogenase at 340 nm with 7.5 mM pyruvate and 0.17 mM NADH (20), and malate dehydrogenase at 340 nm with 2.5 mM cis-aconitate and 0.17 mM NADH (20). Each substrate solution was in 100 mM potassium phosphate buffer (pH 7 or 7.5), and gradient or column fractions (5 to 20 µl) were incubated with 2.5 ml of substrate solution for 10 to 30 min at room temperature. Cytochrome c was assayed by its absorbance at 550 nm. The hydrodynamic parameters of the calibrating enzymes that were used for the calibrating enzymes are in Table I.

**Materials** - Sucrose and (8-¹⁴C)adenosine 3':5'-monophosphate were obtained from Schwarz/Mann. Na⁺, Na⁺⁺, and [α-³²P]ATP were from Amersham/Searle or New England Nuclear. [¹²⁵I]iodohydroxybenzylpindolol was synthesized and purified as described previously (16), and [¹²⁵I]iodohydroxybenzylpindolol was prepared in the same way. The calibrating enzymes, Lubrol PX, and D₂O were from Sigma; Ultrogel was from LKB Instrument Co.; polyethylene glycol was from Fisher; and Bio-Beads SM-2 (90 to 100 mesh) were from Bio-Rad.

**RESULTS**

**Assay of [¹²⁵I]iodohydroxybenzylpindolol** - β-Adrenergic Receptor Complex - Substantial data indicate that [¹²⁵I]iodohydroxybenzylpindolol can be utilized to label β-adrenergic receptor sites selectively (15, 16, 30). Under the conditions employed in this study, 90 to 95% of bound ligand is associated with the receptor when the amount of particulate complex is quantified by filtration through glass fiber filters (15). Since this method is not appropriate for detection of soluble binding sites, a polyethylene glycol precipitation technique (16) was adapted for this purpose. The two procedures were compared for membranes in the presence and absence of detergent (Table II). In the absence of Lubrol PX, the same amount of binding is detected with either technique. This is also true when competition for binding sites by (-) and (+)-propranolol is tested (not shown).

When membranes were treated with Lubrol PX, most (85%) specifically bound [¹²⁵I]iodohydroxybenzylpindolol was still detected by the polyethylene glycol method, but the majority of this binding was lost when glass fiber filters were used (Table II). In the absence of detergent, centrifugation at 12,000 × g for 30 min removed most specifically bound [¹²⁵I]iodohydroxybenzylpindolol. In the presence of Lubrol PX, however, 85% of the bound counts could still be recovered from the supernatant following precipitation with polyethylene glycol. Treatment with trypsin resulted in the loss of most binding to particulate preparations that could be detected by the precipitation method, and no binding was found after the detergent-treated preparation was incubated with the proteolytic enzyme.

The rate of dissociation of [¹²⁵I]iodohydroxybenzylpindolol from particulate β-adrenergic receptor sites is 0.004 min⁻¹ at 30°C (15), consistent with the amount of ligand lost during incubation of control membranes in the absence of trypsin (Table II). Considerably more ligand is lost from the detergent-treated preparation when it is incubated at 30°C. It is not known if this rate represents true dissociation or if the recep-

<table>
<thead>
<tr>
<th>Protein</th>
<th>Partial specific volume, λ</th>
<th>Sedimentation coefficient, s₂₀,w</th>
<th>Diffusion coefficient, D₆₅₀</th>
<th>Stokes radius, a*</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-Galactosidase (21)</td>
<td>0.76</td>
<td>15.93</td>
<td>3.12</td>
<td>6.84</td>
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<td>Fumarase (22-24)</td>
<td>0.738</td>
<td>9.09</td>
<td>4.05</td>
<td>5.27</td>
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<tr>
<td>Catalase (25)</td>
<td>0.70</td>
<td>11.3</td>
<td>4.10</td>
<td>5.21</td>
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<tr>
<td>Lactate dehydrogenase (26)</td>
<td>0.74</td>
<td>7.3</td>
<td>4.5</td>
<td>4.75</td>
</tr>
<tr>
<td>Malate dehydrogenase</td>
<td>0.74</td>
<td>4.32</td>
<td>5.78</td>
<td>3.69</td>
</tr>
<tr>
<td>Cytochrome c (29)</td>
<td>0.728</td>
<td>1.71</td>
<td>11.4</td>
<td>1.87</td>
</tr>
</tbody>
</table>

* The values for α were calculated from the diffusion coefficient according to the equation a = kT/6πηs₂₀,wD₆₅₀, where k is the Boltzmann constant, η is the viscosity of water at 20°C. ** The values for lactate dehydrogenase are an average of those for the protein from bovine heart and from chicken muscle. These differ little from each other or from other sources of the enzyme. Parameters have not been determined for the rabbit muscle protein.

**The diffusion coefficient for malate dehydrogenase was calculated using the value for the molecular weight (70,000) from Ref. 28 and the value of s₂₀,w from Ref. 27.**
The same concentration of (+)-propranolol has little effect. The inhibited almost completely by 10 nM (-)-propranolol; the was used and 270 cpm when the polyethylene glycol precipitation technique was employed. These values have been subtracted from the counts per min for samples incubated in the absence of propranolol.

Numerous attempts were made to bind [125I]iodohydroxybenzylpindolol to receptor sites after treatment of membranes with various detergents, including digitonin. The latter compound has been used successfully to solubilize the receptor from frog erythrocyte membranes (11). None of these experiments was successful, including a few [3H]dihydroalprenolol, rather than [125I]iodohydroxybenzylpindolol, was the ligand. Experiments were performed with various detergents, and several temperatures and times of incubation were tested. Attempts were also made to "stabilize" the receptor with readily dissociable antagonist or agonist ligands during solubilization, followed by removal of ligands or detergent, or both, prior to incubation with [125I]iodohydroxybenzylpindolol. While we feel that some technique should be successful, the appropriate condition is yet to be found.

Since soluble binding sites can thus be characterized only by virtue of the ligand already bound to them, further substantiation is required of their identity with the membrane-bound sites characterized previously (10). The β-adrenergic receptor binds ligands with a high degree of stereoselectivity, and binding of [125I]iodohydroxybenzylpindolol to membranes is inhibited almost completely by 10 nm (-)-propranolol; the same concentration of (+)-propranolol has little effect. The presumed ligand-receptor complex should therefore be solubilized from membranes incubated with radioactive ligand in the presence of 10 nm (+)-propranolol, but not when the biologically active enantiomer is present. Fig. 1 shows the hydrodynamic behavior of bound (polyethylene glycol-precipitable) [125I]iodohydroxybenzylpindolol obtained by treatment of membranes with Lubrol PX after incubation with radioactive ligand in the presence of (-)- or (+)-propranolol. When membranes were incubated with radioactive ligand in the presence of (+)-propranolol, clear peaks of bound [125I]iodohydroxybenzylpindolol are observed during gel filtration (Fig. 1A) or sucrose density gradient centrifugation (Fig. 1B). No peak of...
bound ligand is observed when a low concentration of (−)-propranolol was present during the ligand binding reaction. Thus, virtually all [125I]iodohydroxybenzylpindolol bound to the membranes meets criteria for binding to the β-adrenergic receptor (15), and most of this can be recovered in the Lubrol supernatant. It is therefore most reasonable to assume that the major peaks observed in Fig. 1 are in fact the true receptors. The alternative would be to postulate the quantitative transfer of bound ligand from the receptor to another macromolecule with high affinity for the ligand during solubilization. This seems very unlikely, particularly since essentially no binding of [125I]iodohydroxybenzylpindolol could be obtained in the presence of Lubrol PX and since such a hypothetical binding site is not obvious when membranes are studied with this rather hydrophobic ligand.

Fig. 1A also shows the behavior of free [125I]iodohydroxybenzylpindolol applied to the Ultrogel column; the radioactive ligand appears well in advance of the point of elution of dinitrophenol glycine. This is presumably due to association of the ligand with detergent micelles. A minor fraction of ligand (not incubated with receptor) is detected with the polyethylene glycol precipitation technique in a peak behind that for the ligand-receptor complex. The data obtained with (+)- and (−)-propranolol clearly distinguish this peak from that of the presumed ligand-receptor complex.

Gel Filtration—The elution patterns of adenylate cyclase and bound [125I]iodohydroxybenzylpindolol from the Ultrogel column are shown in Fig. 2A, and they are clearly distinguishable. The Stokes radius for the enzyme is estimated to be 7.1 nm while that for the receptor is 6.4 nm. These values were obtained from a standard curve of distribution coefficient (Kd) versus Stokes radius, constructed for the calibrating enzymes (Fig. 2B). Values of 7.4 and 6.4 nm were obtained if a standard curve of log Kd versus Stokes radius was used; a linear relationship was not found between Kd and the Stokes radius (31). The elution pattern was not altered by the addition of 10% sucrose to the Lubrol solution used to equilibrate and elute the column (see Ref. 32). Small peaks of both receptor and enzyme appear in the void volume, these, perhaps,
Sucrose Density Gradient Centrifugation—Fig. 3, A and B shows the results of centrifugation through gradients of 5 to 20% sucrose in H$_2$O or D$_2$O. The peak of adenylate cyclase activity is well separated from that of polyethylene glycol-precipitable $[^{125}]$iodohydroxybenzylpindolol. A smaller variable peak of adenylate cyclase activity is sometimes seen; this sediments near the peak of receptor in H$_2$O and remains at the meniscus in D$_2$O. This could correspond to a component with high partial specific volume—perhaps hypothetical membrane fragments that appear in the void volume during gel filtration (Fig. 2).

When Lubrol PX was partially removed by treatment with Bio-Beads SM-2, adenylate cyclase activity and bound $[^{125}]$iodohydroxybenzylpindolol were found in very broad peaks near the position of $\beta$-galactosidase (in H$_2$O). This is presumably due to the formation of aggregates that follows removal of the detergent.

Calculation of Molecular Parameters—Martin and Ames (33) showed that there should be a very nearly linear relationship between $s_{20,w}$ and the distances traveled by proteins in 5 to 20% sucrose gradients, unless the proteins differ in partial specific volume. This was the case for the calibrating enzymes used for the gradients in D$_2$O as well as in H$_2$O (Fig. 4). If adenylate cyclase and the ligand-receptor complex have values of partial specific volume similar to those of the calibrating enzymes, the same experimentally determined sedimentation coefficients should be obtained in H$_2$O and D$_2$O. This was not the case. For adenylate cyclase the experimental sedimentation coefficient was 7.3 ± 0.3 S in H$_2$O and 6.5 ± 0.2 S in D$_2$O, the corresponding values for the $[^{125}]$iodohydroxybenzylpindolol-receptor complex were 2.7 ± 0.3 S and 1.4 ± 0.2 S. These data indicate that the partial specific volumes of both proteins in detergent solution are larger than those of the calibrating enzymes. The most likely explanation for this fact is that detergent is bound to each protein.

Calculation of partial specific volume and $s_{20,w}$ was carried out by combining the data for centrifugation in H$_2$O and D$_2$O (35, 36). Values of molecular weight were calculated from $s_{20,w}$, partial specific volume, and the Stokes radius (Table III). The hypothetical amount of bound detergent and the molecular weight of the protein portion of the detergent-protein complex were estimated with the assumption that the partial specific volume represents the average of protein (0.71 to 0.76 ml/g) and Lubrol PX (0.958 ml/g). Calculations are summarized in Table III. It was also assumed that the true values of $s_{20,w}$ and partial specific volume are independent of the solvent. This is not strictly valid, since proteins may bind different amounts of detergent in H$_2$O and D$_2$O (23, 36). However, the error inherent in this assumption appears to be small (36).

TABLE III

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Adenylate cyclase</th>
<th>$\beta$-Adrenergic receptor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stokes radius, $\bar{a}$ (nm)</td>
<td>7.1 ± 0.04 (3)</td>
<td>6.4 ± 0.03 (3)</td>
</tr>
<tr>
<td>Partial specific volume, $\bar{v}$ (ml/g)$^a$</td>
<td>0.78 ± 0.01 (7 × 6)</td>
<td>0.83 ± 0.01 (7 × 6)</td>
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<tr>
<td>Sedimentation coefficient, $s_{20,w}$ (S)$^a$</td>
<td>7.5 ± 0.3 (7 × 6)</td>
<td>3.1 ± 0.3 (7 × 6)</td>
</tr>
<tr>
<td>Molecular weight, $M_r^c$</td>
<td>270 × 10$^3$</td>
<td>130 × 10$^3$</td>
</tr>
<tr>
<td>Frictional ratio, $f/f_0^c$</td>
<td>1.6</td>
<td>1.8</td>
</tr>
<tr>
<td>Lubrol PX bound$^d$ mg/mg protein</td>
<td>0.2 (0.4–0.1)</td>
<td>0.8 (1.0–0.6)</td>
</tr>
<tr>
<td>mol/mol protein</td>
<td>90 (120–40)</td>
<td>100 (110–80)</td>
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<tr>
<td>Molecular weight of protein$^a$</td>
<td>220 × 10$^3$ (200–250 × 10$^3$)</td>
<td>75 × 10$^3$ (68–85 × 10$^3$)</td>
</tr>
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$^a$ The values given are the mean ± 1 S.D. for the number of determinations shown in parentheses.

$^b$ Seven sucrose gradient centrifugations were done in H$_2$O and six were done in D$_2$O. The values of $\bar{v}$ and $s_{20,w}$ given are the mean ± 1 S.D. calculated for the 42 combinations of gradients.

$^c$ Molecular weights were calculated according to the following equation:

$$M_r = \frac{6\pi N\eta_{20,w}}{1 - \delta \rho_{20,w}} \cdot a \cdot s_{20,w},$$

where $N$ is Avogadro's number, $\eta_{20,w}$ is the viscosity of water at 20°, and $\rho_{20,w}$ is the density of water at 20°.

$^d$ Frictional ratios were calculated according to the following equation:

$$f/f_0 = (4\pi N/3M_r)^{1/3}.$$
**Table IV**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Wild type</th>
<th>Clone</th>
<th>AC</th>
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<tr>
<td>Adenylate cyclase</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Stokes radius (nm)</td>
<td>7.1 ± 0.04 (3)</td>
<td>7.2 (1)</td>
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<tr>
<td>Partial specific volume (ml/g)</td>
<td>0.78 ± 0.01 (7 x 6)</td>
<td>0.78 ± 0.01 (2 x 2)</td>
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</tr>
<tr>
<td>( s_{\text{pol}} ) (SI)</td>
<td>7.5 ± 0.3 (7 x 6)</td>
<td>7.8 ± 0.3 (2 x 2)</td>
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<tr>
<td>( \beta )-Adrenergic receptor</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stokes radius (nm)</td>
<td>6.4 ± 0.03 (3)</td>
<td>6.4 (1)</td>
<td>6.2 (1)</td>
</tr>
<tr>
<td>Partial specific volume (ml/g)</td>
<td>0.83 ± 0.01 (7 x 6)</td>
<td>0.82 ± 0.01 (2 x 2)</td>
<td>0.84 ± 0.01 (2 x 2)</td>
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<tr>
<td>( s_{\text{pol}} ) (SI)</td>
<td>3.1 ± 0.3 (7 x 6)</td>
<td>2.8 ± 0.4 (2 x 2)</td>
<td>3.4 ± 0.3 (2 x 2)</td>
</tr>
</tbody>
</table>

* The values given are the mean ± 1 S.D. for the number of determinations shown in parentheses.

**Variant S49 Lymphoma Clones**—Gel filtration and sucrose density gradient centrifugation were carried out for the ligand-receptor complex from a cell clone that lacks adenylate cyclase (AC) (14), and both the receptor and adenylate cyclase were examined in a clone that has both functions but where ligand binding to the receptor causes no enzyme activation (UNC) (8). Calculated parameters were very similar to those determined for receptor and adenylate cyclase from the wild type clone (Table IV). To compare the receptor from wild type cells and UNC cells more carefully, the complex of wild type receptor and \([\text{I}]\text{Iodohydroxybenzylpindolol} \) was mixed with the complex of UNC receptor and \([\text{I}]\text{Iodohydroxybenzylpindolol} \). The labeled receptors sedimented together through sucrose density gradients (not shown). At this relatively crude level of observation, therefore, the reason responsible for the UNC phenotype is not assignable to any discernable difference in receptor or enzyme.

**DISCUSSION**

Adenylate cyclase activity and a complex of \([\text{I}]\text{Iodohydroxybenzylpindolol} \) with the \( \beta \)-adrenergic receptor can be resolved into distinct peaks by either gel filtration or sucrose density gradient centrifugation. The two functions thus reside on noncovalently linked structures. This observation is consistent with the existence of variants that are deficient in one function (7, 14) or in which the two functions are uncoupled (8). Selective ablation of either function can also be achieved chemically (4, 5), and ligand binding can be uncoupled from enzyme activation by treatment with certain phospholipases (37) or by filipin (38). The observations reported herein and those of Orly and Schramm (4) and Limbird and Lefkowitz (12) are not consistent with the model of Stellwagen and Baker (39), who proposed that adrenergic ligand binding sites and adenylate cyclase catalytic sites are on the same peptide chain.

The data reported above are similar to those obtained previously by Neer for adenylate cyclase from the renal medulla of the rat (15). However, the values in this report for molecular weight and for partial specific volume are somewhat greater. Neer was unable to detect significant binding of detergent to adenylate cyclase by the same techniques used in this study. The reasons for these discrepancies are not completely clear. However, Neer has recently reported a larger species of the enzyme from canine renal medulla (40), and hydrodynamic parameters for the brain enzyme are nearly identical to the values above for the S49 cell protein. The enzyme from different cells and species may obviously differ; the enzyme may be solubilized in different states, dependent on conditions; or the rat renal medullary enzyme as studied may have been a proteolytic fragment, from which a hydrophobic pedicle was cleaved.

Hydroporphic regions on the surface of a protein may bind detergent micelles or they may be covered by detergent monomer, or both (41). Based on the assumptions discussed above, the number of molecules of Lubrol PX bound to adenylate cyclase and to the ligand-receptor complex have been calculated (Table III). In each case, the amount of detergent approximates that of a single micelle (106 molecules) (41). This may, of course, be coincidence, and further work will be necessary to distinguish the possibilities.

Since detergent binding to adenylate cyclase was not apparent previously, Neer proposed that there was minimal penetration of the membrane by the enzyme. The receptor would then need to span the bilayer to interact with adenylate cyclase, and a high value of partial specific volume was thus predicted (13). The data of this report are consistent with such a model. Since a higher value of partial specific volume has now been found for adenylate cyclase, the data are also consistent with models that envision penetration of both species into the bilayer, and the two proteins could interact in this manner. However, since occupation of one receptor by agonist ligands appears to lead to an extremely variable rate of synthesis of cyclic AMP when different systems are compared, we have recently suggested that the stoichiometry of interaction between receptor and enzyme may vary over a wide range (42). This lends some credence to the possibility that the receptor and the enzyme may be integral membrane proteins that do not interact directly with each other but that communicate by mechanisms that are less subject to stringent stoichiometric restraints.

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