Solubilization of Prostacyclin Membrane Receptors from Human Platelets*

Ah-lim Tsai†, Ming-Jo Hsu, Hadassah Vijeswarapu, and Kenneth K. Wu

From the Department of Internal Medicine, Division of Hematology/Oncology and the Center for Vascular and Thrombosis Research, The University of Texas Health Science Center, Houston, Texas 77030

Prostacyclin (PGI₂) receptors have been identified on platelets and other tissues but their physiochemical properties remain unknown due to difficulties in obtaining active solubilized receptors. We evaluated the ability of several detergents to release the receptors from platelet membrane preparations. In contrast to the results of Dutta-Roy and Sinha (Dutta-Roy, A. K., and Sinha, A. K. (1987) J. Biol. Chem. 262, 12685–12691) which revealed selective solubilization of PGE₁/PGI₂ receptors by 0.05% Triton X-100, we found that CHAPS (3-

D-(3-cholamidopropyl)dimethylammonio]-1-propanesulfonic acid) (10 mM) was far superior in releasing the PGI₂ receptors. In fact, Triton X-100 failed to release detectable PGI₂ binding activity into the supernatant. The CHAPS-solubilized receptor degraded rapidly unless 30% glycerol was added which greatly enhanced its stability. By employing an improved binding assay using [³H]iloprost as the ligand and selective membrane filters (AP-15 or GF/B) pretreated with polyethyleneimine for achieving a higher trapping efficiency, we showed by equilibrium binding measurements that the solubilized receptors exhibited a single class of binding sites with a dissociation constant of 18.5 nM and B₅₀, 0.5 pmol/mg. These values were similar to those of the membrane receptors, i.e. Kᵈ of 18.6 nM and B₅₀, 1.0 pmol/mg. Kinetic binding measurements of the solubilized receptors revealed an association rate constant of 0.51 × 10⁶ M⁻¹ s⁻¹ and dissociation rate constant of 0.0041 s⁻¹ yielding a calculated Kᵈ of 8.0 nM. Displacement of [³H]iloprost (Kᵈ values) from the solubilized and the membrane receptors by diversified eicosanoids was parallel. Our data demonstrate for the first time a successful solubilization of platelet PGI₂ receptors. The solubilized receptors retained almost identical binding characteristics as the native membrane receptors.

Although PGI₂ receptors have been identified on platelets, vascular tissues, lung tissues, and neuronal hybrid cells (4–7), their physiochemical characteristics, including the binding kinetics, remain uncertain because of several inherent problems including chemical instability of PGI₂ (8), difficulty in obtaining active solubilized receptors, and lack of reliable binding assays (9). Leigh et al. (10) provided the first information on the identity of platelet PGI₂ receptors using the radiation inactivation technique. They reported an estimated molecular weight of 82,800. They were, however, unable to solubilize the PGI₂ receptors without losing activity. Dutta-Roy and Sinha (11), on the other hand, reported solubilization of a PGE₁ receptor from human platelets by 0.05% Triton X-100 and purification of the receptor protein to homogeneity from the solubilized fraction. Based on the data of binding displacement and reconstitution, they proposed that the purified receptor is also the receptor for PGI₂. Using iloprost, a stable prostacyclin analog as a ligand for binding studies, we did not detect binding activity in the Triton X-100-treated supernatant fraction. In contrast, we found that CHAPS was far superior in releasing the receptors into the supernatant. By protecting the stability of the receptor with 30% glycerol, we further showed that the solubilized receptors essentially maintained identical binding characteristics as the native membrane receptor.

MATERIALS AND METHODS

RESULTS

Search for Optimum Conditions to Solubilize the PGI₂ Receptors—We tested five different detergents for their capacity to solubilize PGI₂ receptors for platelet membranes. Detailed results are shown in the Miniprint Section. 0.05% Triton X-100 did not solubilize any significant amount of PGI₂ receptors. Instead, we found that the use of 10 mM CHAPS containing 30% glycerol was optimal for solubilizing the platelet PGI₂ receptor proteins. Moreover, treatment of certain selected membrane filters such as GF/B and AP-15 with polyethyleneimine increased the trapping efficiency and facilitated the assay for binding parameters. At this detergent concentration, we solubilized approximately half of the membrane-associated protein and also about half of the total receptor activity suggesting a nonselective solubilization of PGI₂ receptors by CHAPS.

Binding Characteristics of Solubilized PGI₂ Receptors Using [³H]Iloprost as Ligand—As shown in Fig. 3, the solubilized

*This work was supported by National Institutes of Health Grants P61 NS-18494 and NS-23327. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

†To whom correspondence should be addressed: Dept. of Internal Medicine, Div. of Hematology/Oncology, the University of Texas Health Science Ctr., P. O. Box 20708, Houston, TX 77225.

The abbreviations used are: PG, prostaglandin; CHAPS, 3-

Solubilization of Platelet PGI<sub>2</sub> Receptors

Receptor had an identical ligand concentration dependence for its binding activity as the membrane-associated receptors. Binding data were fit nicely to a one binding class model by nonlinear regression analysis. \( K_D \) was determined to be 18.5 and 16.6 nM for the solubilized and membranous receptors, respectively. Solubilization of platelet membrane receptor proteins by CHAPS apparently did not cause any damage to their binding affinity for PGI<sub>2</sub>. The total binding capacity of the solubilized receptors was approximately half of the membranous receptors (0.50 pmol/mg versus 1.0 pmol/mg). This is expected since the membrane filters used in the binding assay exhibited about 60% efficiency and the release of the membrane proteins by CHAPS was nonselective.

The interaction between PGI<sub>2</sub> and its receptor was further characterized by the kinetic binding measurements. The association of ["H"]iloprost with the solubilized receptors was dependent on the ligand concentration. At each ligand concentration, binding follows a pseudo first-order process (Fig. 4A). The rate constant determined by linear regression of the observed pseudo first-order rates was \( 0.51 \times 10^6 \text{ M}^{-1} \text{ s}^{-1} \) (inset of Fig. 4A) at 25 °C. Dissociation of ["H"]iloprost from the ligand-receptor complex was a first-order process and was completed in less than 10 min (Fig. 4B). The dissociation rate constant was determined to be 0.0041 s<sup>-1</sup> at 25 °C by logarithmic transformation (inset of Fig. 4B). From these data, \( K_D \) was calculated to be 8.0 nM, a value lower than that obtained by binding equilibrium method, i.e. 18.5 nM.

Displacement of ["H"]iloprost by Various Eicosanoids—As shown in Fig. 5, detergent-solubilized and membrane-associated receptors displayed almost identical behaviors in ligand displacement. Specific ["H"]iloprost binding to the platelet receptors is saturable and fully reversible. Iloprost, carbacyclin, PGE<sub>1</sub>, and 6β-PGI<sub>1</sub> completely displaced ["H"]iloprost to the level of nonspecific binding and each displacement curve was satisfactorily fit by a simple competitive model (detailed in the legend of Fig. 5). \( K_i \) values for iloprost, carbacyclin, PGE<sub>1</sub>, and 6β-PGI<sub>1</sub> were 22.8, 528, 1,490, and 61,500 nM, respectively, for the solubilized receptors and were 25, 518,
Solubilization of Platelet PGI₂ Receptors

3,300, and 41,700 nM, respectively, for the membranous receptors. PGD₂, which was considered to have different platelet receptors (5, 18) failed to show any competition of binding. The authentic ligand, PGI₂ exhibited significant displacement but was weaker than iloprost in the displacement of [³H]iloprost (Kᵢ values were 435 and 419 nM for the membranous and solubilized receptors, respectively). The disparity is due to the chemical instability of PGI₂ (3, 8). When we corrected the displacement data for the chemical hydrolysis of PGI₂ using a half-life of 3.5 min as calculated by Cho and Allen (8), the corrected values of Kᵢ were 22.3 and 21.5 nM for the membranous and solubilized receptors, respectively. These values are comparable to those using iloprost as the displacing ligand. Because the thromboxane A₂/prostaglandin H₂ receptor has been solubilized by a procedure similar to that of PGI₂ receptors (19, 20), we also tested 13-azaprostanoic acid (an agonist of TXA₂/PGH₂ receptor) (21) and U46619 (an agonist of thromboxane A₂/PGH₂ receptor) (22) for their displacement activity. Neither compounds displaced [³H]iloprost from the solubilized receptors (Fig. 5). Their competition effects were similar to PGD₂ displacement. By contrast,
Solubilization of Platelet PGI\textsubscript{2} Receptors

FIG. 5. Displacement of [\textsuperscript{3}H]iloprost binding from membranous and solubilized PGI\textsubscript{2} receptors by various eicosanoids. Displacement experiments were performed at room temperature with 24 nM [\textsuperscript{3}H]iloprost and serial concentrations of various ligands. $\square$, iloprost; $\square$, PGI\textsubscript{2}; $\Delta$, carbacyclin; $+$, PGE\textsubscript{1}; $*$, 6-k-PGI\textsubscript{2}; $\times$, PGD\textsubscript{2}; $+$, U46619; and $\bullet$, 19-APA. Inhibition constants ($K_i$) were determined from: $B/B_0 = ([[L] + K_0)/([L] + K_0(1 + [I]/K_i))$ by nonlinear regression analysis where $B$ and $B_0$ indicate bound [\textsuperscript{3}H]iloprost in the presence and absence of competing ligands, $[L] = 23.8$ nM, and $K_0$ is the dissociation constant; this value is 18.6 nM for membranous receptors and 18.5 nM for solubilized receptors. Each displacement curve was displayed as normalized displaceable binding relative to the control which did not contain any displacing ligand.

FIG. 6. Specific PGI\textsubscript{2} binding of the platelet membrane ($\bigcirc$), the membranous (+) and supernatant (\textdegree) fractions obtained after 0.05% Triton X-100 treatment and centrifugation. $K_0$ for these three samples was estimated to be 9.4, 6.5, and 19.4 nM and $B_{\text{max}}$ 0.20, 0.21, 0.016 pmol/0.4 mg of protein, respectively. Values were obtained by nonlinear regression to a model involving one binding species.

they enhanced [\textsuperscript{3}H]iloprost binding to the membrane-associated receptors.

**DISCUSSION**

In the present work we demonstrated successful solubilization of PGI\textsubscript{2} receptors from the platelet membrane by using CHAPS. The solubilized receptors remained in the supernatant after centrifugation at 150,000 $\times$ g for 1 h. The binding characteristics of the solubilized receptors are almost identical to those of the membrane-associated receptors measured by kinetic and equilibrium binding and binding displacement experiments. Despite multiple attempts by utilizing various concentrations of Triton X-100 and platelet preparations, we were unable to reproduce the results of Dutta-Roy and Sinha (11) on the selective release of PGE\textsubscript{1}/PGI\textsubscript{2} receptors by Triton X-100. The typical binding data conducted on the samples prepared by the procedure of Dutta-Roy and Sinha for solubilizing the receptor proteins with 0.05% Triton X-100 are represented by the binding isotherm shown in Fig. 6. Even though 20% of the membrane proteins were released in the supernatant by Triton X-100, the PGI\textsubscript{2} binding activity was undetectable in the supernatant and all the binding activity remained with the pellet fraction. There was no loss of total binding activity in the pellet. In fact, the $B_{\text{max}}$ was increased slightly coinciding nicely with the loss of nonreceptor proteins after Triton treatment. These results could suggest that Triton X-100 might release PGE\textsubscript{1} receptors without solubilizing PGI\textsubscript{2} receptors. In three trials we failed to release more than 30% of the E\textsubscript{1} receptors by the procedure of Dutta-Roy and Sinha using a platelet membrane preparation and 0.1% Triton despite a 17% release of the total membrane proteins in the supernatant. When whole platelets were used, we could not observe any binding activity in the supernatant fraction after treatment of 0.05% Triton. Our results indicate that Triton X-100 did not appear to solubilize any PGI\textsubscript{2} or significant PGE\textsubscript{1} receptors from human platelets. Solubilization of PGI\textsubscript{2} receptors has unsuccessfully been attempted by another lab-
Solubilization of Platelet PGI2 Receptors

Although many experimental results including this work (Fig. 5) have demonstrated similar binding displacement by PGI2 and PGE2, leading to the suggestion that PGI2 and PGE2 may share the same receptors (18, 23), these data should be interpreted cautiously. First of all, a mutual displacement between PGI2 and PGE2 for their specific receptors does not necessarily mean a direct competition for an identical physical binding site. Although Schiller and Prior (23) have demonstrated by double-reciprocal plot of the binding data a competitive inhibition between PGE2 and iloprost for platelet receptors, the approaches by organic synthesis of stable PGI2 analogs provided convincing evidence that the structural requirements for PGI2-receptor interaction is extremely stringent (28, 29). Given the obvious structural difference between PGI2 and PGE2, it is difficult to envision that these two compounds could occupy the same rigid domain. An alternative explanation is that the PGI2 and PGE2 binding domains are not identical but physically close or even overlap and therefore exhibit mutually exclusive binding.

We have attempted to determine the molecular weight of the solubilized receptors by gel filtration procedures using Sephacryl-300 and TSK G3000SW (LKB UltroChrom GTi Bioporation System). Preliminary data revealed that this solubilized protein has an apparent molecular mass greater than 150 kDa. This value is far greater than the value of 83 kDa estimated by the method of radiation inactivation (10) suggesting that the solubilized receptor might be associated with other macromolecules. We are currently examining the possible association of guanine nucleotide-binding proteins. These G protein(s) were found to be closely coupled with various receptors including PGI2 receptors for signal transduction (30, 31).

In summary, an active PGI2 receptor has been solubilized by CHAPS from the platelet membrane and its ligand binding has been characterized. The binding characteristics of the solubilized receptor is almost identical to that of the membrane-associated receptor using [3H]iloprost as binding ligand. If we assume one molecule of the solubilized receptor using [3H]iloprost as binding ligand. If we assume one molecule of the solubilized receptor binds one molecule of ligand, a receptor density of 743 fmol/mg (12) would require a purification of 10,000-fold to reach homogeneity. This estimation is close to that of the platelet thromboxane A2/PGH2 receptor (19) and other membrane-associated receptors.

Acknowledgement—We wish to thank Nancy Fernandez for preparing this manuscript.

REFERENCES


2 A.-I. Tsai, M.-J. Hsu, H. Yijjeswarapu, and K. K. Wu, unpublished data.
Solubilization of platelet PG12 receptors


SOLUBILIZATION OF PLATELET PG12 RECEPTORS

Pharmacology was performed by neutralizing recrystallized SIgM oxide and adjusting to pH 7.4 using NaOH.

Platelet Membrane Preparation. Washed platelets were prepared from recently expired platelets purchased from a local blood bank (at least 50-100 units for each process) following the procedure previously described (12). The washed platelets were suspended in processing. To prepare crude platelet membranes, the frozen platelets were thawed and processed according to Tsai and Lefkowitz (13).

Solubilization of PG12 Receptors. Crude platelet membrane pellets were resuspended in binding buffer (50 mM Tris-Cl, pH 7.4) and 1.5 M NaCl at pH 7.4 containing 3% glycerol and 10 mM GMP (buffer A) at a concentration of 0.1 mg protein/mL. The protein concentration was determined by both Bradford method (14) and biuret method (15).

RESULTS

To test for differences between the binding buffer and buffer A, the binding activity was measured by a modified binding assay. As shown in Table 1, the activity of buffer A is slightly higher than that of the binding buffer. This difference may be due to the higher concentration of glycerol. Further experiments are needed to determine whether the binding activity is affected by the higher concentration of glycerol. The activity of buffer A was also measured by a modified binding assay. As shown in Table 1, the activity of buffer A is slightly higher than that of the binding buffer. This difference may be due to the higher concentration of glycerol. Further experiments are needed to determine whether the binding activity is affected by the higher concentration of glycerol.
TABLE I

Dispersed binding activity of platelet membranes and its substrates
after solubilization by various detergents

<table>
<thead>
<tr>
<th>SAMPLE</th>
<th>SPECIFIC ACTIVITY (pmol/mg)</th>
<th>TOTAL PROTEIN (mg)</th>
<th>TOTAL ACTIVITY (pmol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EXP. 1. 1% Triton X-100</td>
<td>0.10</td>
<td>1.00</td>
<td>0.10</td>
</tr>
<tr>
<td>EXP. 2. 1% Triton X-100</td>
<td>0.01</td>
<td>1.00</td>
<td>0.01</td>
</tr>
<tr>
<td>EXP. 3. 1% Triton X-100</td>
<td>0.01</td>
<td>1.00</td>
<td>0.01</td>
</tr>
<tr>
<td>EXP. 4. 1% Triton X-100</td>
<td>0.01</td>
<td>1.00</td>
<td>0.01</td>
</tr>
<tr>
<td>EXP. 5. 1% Triton X-100</td>
<td>0.01</td>
<td>1.00</td>
<td>0.01</td>
</tr>
<tr>
<td>EXP. 6. 1% Triton X-100</td>
<td>0.01</td>
<td>1.00</td>
<td>0.01</td>
</tr>
<tr>
<td>EXP. 7. 1% Triton X-100</td>
<td>0.01</td>
<td>1.00</td>
<td>0.01</td>
</tr>
<tr>
<td>EXP. 8. 1% Triton X-100</td>
<td>0.01</td>
<td>1.00</td>
<td>0.01</td>
</tr>
<tr>
<td>EXP. 9. 1% Triton X-100</td>
<td>0.01</td>
<td>1.00</td>
<td>0.01</td>
</tr>
</tbody>
</table>

TABLE II

Dispersed binding activity of the supernatant fractions after detergent

<table>
<thead>
<tr>
<th>SAMPLE</th>
<th>w/o PEI Treatment (pmol/mg)</th>
<th>w/o PEI Treatment (pmol/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.01% Triton</td>
<td>0.01</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>0.1% Triton</td>
<td>0.01</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>1% Triton</td>
<td>0.01</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>10 mM CHAPS</td>
<td>0.01</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>30 mM CHAPS</td>
<td>0.01</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>1% Cholate</td>
<td>0.01</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>1% Cholate</td>
<td>0.01</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

TABLE III

Dispersed binding activity of various PEI-treated glass fiber filters

<table>
<thead>
<tr>
<th>Method</th>
<th>Total</th>
<th>Monospecific</th>
<th>Specific</th>
</tr>
</thead>
<tbody>
<tr>
<td>PEI filtration</td>
<td>1950</td>
<td>765 ± 30</td>
<td>2315 ± 92</td>
</tr>
<tr>
<td>PEI filtration</td>
<td>1950</td>
<td>765 ± 30</td>
<td>2315 ± 92</td>
</tr>
<tr>
<td>PEI filtration</td>
<td>1950</td>
<td>765 ± 30</td>
<td>2315 ± 92</td>
</tr>
<tr>
<td>PEI filtration</td>
<td>1950</td>
<td>765 ± 30</td>
<td>2315 ± 92</td>
</tr>
<tr>
<td>PEI filtration</td>
<td>1950</td>
<td>765 ± 30</td>
<td>2315 ± 92</td>
</tr>
<tr>
<td>PEI filtration</td>
<td>1950</td>
<td>765 ± 30</td>
<td>2315 ± 92</td>
</tr>
<tr>
<td>PEI filtration</td>
<td>1950</td>
<td>765 ± 30</td>
<td>2315 ± 92</td>
</tr>
<tr>
<td>PEI filtration</td>
<td>1950</td>
<td>765 ± 30</td>
<td>2315 ± 92</td>
</tr>
</tbody>
</table>

*Note: Values are means of triplicate measurements. 0.2 mg CNSP-solubilized PGI2 receptors
were incubated with 25 μl (1) PEI solution before assay measurement.

Procedure of the PEI-filtration was detailed under Methods and Materials.