Purification of A₁ Adenosine Receptor from Rat Brain Membranes*

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The A₁ adenosine receptor from rat brain membranes has been purified about 50,000-fold to apparent homogeneity by sequential use of affinity chromatography on immobilized xanthine amine congener-agarose, hydroxyapatite chromatography, and reactivity chromatography. The overall yield starting from the membranes was approximately 4%. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of the purified preparation gave a broad single band of an apparent molecular weight of 34,000 either by silver staining or autoradiogram after radioiodination. The purified receptor bound approximately 24 nmol of 8-cyclopentyl-1,3-[3H]dipropylxanthine/mg of protein with a dissociation constant of 1.4 nM. This maximum specific binding value is consistent with the expected theoretical specific activity (29.4 mmol/mg) for a protein with a molecular mass of 34,000 daltons if it is assumed that there is one ligand-binding site/receptor molecule. After extensive washing with dimethyl sulfoxide, and the moist gel containing 100 mg of XAC. The gel suspension was incubated at room temperature overnight with continuous rotation. The reaction was stopped by washing the gel with dimethyl sulfoxide extensively. The gel was further washed sequentially with 1 M Tris, 1 M Tris, and 1 M Tris acetate buffer, pH 7.2, for 24 h at 4 °C. Finally, after washing with water, the XAC-agarose was stored at 4 °C in 0.02% NaN₃. The amount of covalently bound XAC was estimated to be about 1.8 μmol/ml of gel by monitoring the absorbance at 310 nm in 0.01 N HCl of the starting XAC solution and the wash filtrate. Membrane Preparation and Solubilization—Brain membranes were prepared from rat whole brains as described (13) and stored at −85 °C. Frozen membranes were thawed and resuspended in 50 mM Tris acetate buffer, pH 7.2, containing 1 mM EDTA and 5 mM MgCl₂ and centrifuged at 38,000 × g for 25 min. The washed membranes (about 80 g, wet weight) were solubilized in 1,000 ml of 50 mM Tris acetate buffer, pH 7.2, containing 1% digitonin, 0.1% sodium cholate, 1 mM EDTA, 5 mM MgCl₂, 100 mM NaCl, 1 mM dithiothreitol, 0.1 mM phenylmethylsulfonyl fluoride, and 1 μg/ml each of peptatin A, leupeptin, chymostatin, and antipain. The suspension was stirred on ice for 1 h and then centrifuged at 100,000 × g for 1 h at 4 °C. The supernatant was saved as the solubilized preparation and was used immediately for further purification.

Purification of the A₁ Adenosine Receptor—All the purification procedures were performed at 4–8 °C. The solubilized preparation (1000 ml) was applied on an XAC-agarose column (2.5 × 14 cm) at a flow rate of 50 ml/h. The pass-through fractions were saved and used as an additive to the binding assay mixture as described later. The column was then washed with 50 ml of 50 mM Tris acetate buffer, pH 7.2, containing 100 mM NaCl, 1 mM EDTA, and 0.1% digitonin (buffer A). The bound receptor was eluted with 3

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1 The abbreviations used are: XAC, xanthine amine congener, 8-[4-[[1,2-aminoethyl]amino]carbonyl]methyl]oxy]phenyl]-1,3-dipropylxanthine; DPCPX, 8-cyclopentyl-1,3-dipropylxanthine; CPT, 8-cyclopentyltheophylline; DIO-DPCPX, 1,4-phenoxydiisothiocyanate; DITC-XAC, 1,3-dipropyl-8-(isothiocyanatophenyl)aminomethylcarboxylic acid; DTT, dithiothreitol; EDC, N-hydroxy-succinimide ester; HEPES, N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid.
volumes of buffer A containing 100 μM CPT at a flow rate of approximately 15 ml/h. The active elute (~40 ml) from the XAC-agarose chromatography was applied to a 0.5-ml column of hydroxylapatite at a flow rate of 20 ml/h. The column was washed with 5 ml of buffer A and was eluted successively with 10 ml of 10, 110, and 500 mM potassium phosphate buffer, pH 7.0, each containing 100 mM NaCl and 0.1% digitonin. The eluate was collected for 10 ml each, and the phosphate buffer was diluted 2-fold by the addition of buffer A and then applied to an XAC-agarose column (1 x 5 cm) at a flow rate of 10 ml/h. After washing with 6 column volumes of buffer A, the column was eluted with 1.5-2 volumes of buffer A containing 100 μM CPT. In order to assay the eluted activity from the affinity column, the eluate was desalted on a Sephadex G-50 column (6.6 x 13.5 cm) to separate unbound ligand from the receptor (15). The purified receptor preparation was stored at -85°C for 1-2 weeks without a significant loss of [3H]DPCPX binding.

**Gel Permeation Chromatography**—The purified A1 adenosine receptor (100 μl) concentrated by CentriToc 10 (Amicon) was chromatographed on a TSK-3000 SW (7.5 x 300 mm) steric exclusion column at a flow rate of 0.35 ml/min at 4°C. The mobile phase consisted of 20 mM sodium phosphate buffer, pH 6.8, 150 mM NaCl, and 0.2% digitonin. Fractions of 0.4 ml each were collected, and aliquots (100 μl) from each fraction were used for receptor-binding assays. The remaining portion of the fractions was lyophilized and used for SDS-PAGE. The column was standardized with reference proteins of known molecular weight and Stokes radii.

**Binding Assays**—The binding assays of solubilized and affinity-purified receptor preparations were performed as follows. [3H]DPCPX and receptor preparations were incubated at 0°C for 10-12 h in a 0.25 ml total volume of 40 mM Tris acetate buffer, pH 7.2, 80 mM NaCl, 4 mM MgCl2, and 0.08% digitonin. The binding assays of membrane preparations were performed in the same conditions except without digitonin. For the assay of the preparation after the XAC-affinity chromatography, pass-through fractions of proteins passed through, although 80% of [3H]DPCPX-binding activity remained in the column as shown in Fig. 1A. Extensive washing of the column resulted in little loss of activity. The [3H]DPCPX-binding activity was eluted specifically by CPT, a potent A1 adenosine receptor antagonist. The elution of the binding activity was relatively sharp, and more than 90% of the eluted activity was recovered in the column volume (70 ml) as shown in Fig. 1B. This affinity chromatography step resulted in a 2,500-fold purification over the solubilized preparation with a yield of 40%. The affinity-purified preparation was then applied to a small hydroxylapatite column. Most of the binding activity was adsorbed to this column and eluted with 500 mM potassium phosphate buffer after washing extensively with low concentrations of phosphate buffer as shown in Fig. 1C. By this hydroxylapatite chromatography, the A1 adenosine receptor preparation was not only approximately 10-fold purified but also highly concentrated (14-fold). It should be noted that the adenosine antagonist, CPT, present in the eluates of the XAC-affinity chromatography was removed from the receptor preparation during the hydroxylapatite chromatography. The final step for the purification of the A1 adenosine receptor was re-affinity chromatography of the hydroxylapatite eluate on XAC-agarose as shown in Fig. 1D. After washing with buffer A, the small XAC-agarose column was eluted with 100 μM CPT. More than 50% of the applied binding activity was eluted in the first 5 ml of the elution buffer. The results obtained from a typical A1 adenosine receptor purification are summarized in Table I. The receptor was purified approximately 50,000-fold with an overall yield of approximately 4% of the initial binding in intact membranes.

**Purity and Identity of the Receptor Protein**—Fig. 2 shows the silver-stained pattern of SDS-PAGE of the receptor preparations from various stages of purification. After the second affinity chromatography on the XAC-agarose, a single protein band of M, = 34,000 ± 1000 (n = 6) was revealed (Fig. 2A, lane C). This major band always appeared to migrate as a broad band in SDS gels, and this might indicate microheterogeneity of the receptor moiety. The very minor bands of M, = 97,000 and 29,000 were sometimes observed, but the patterns of these minor bands were inconsistent and not proportional to the binding activity of the samples applied. SDS-PAGE of the purified receptor under denaturing conditions did not alter its migration rate (Fig. 2A, lane D).

In order to determine that the peptide band of M, = 34,000 contains the A1 adenosine ligand-binding site, an aliquot of the purified preparation was labeled with [3H]DITC-XAC, a high affinity acylating antagonist for the A1 adenosine receptor (17), and analyzed by SDS-PAGE. Fluorography of the gel showed a major labeled band at M, = 34,000 coincident with the silver-stained protein band (Fig. 2B, lanes A-D).
Purification of $A_1$ Adenosine Receptor

All the data in this table are from one preparation that is representative of six similar experiments that have been done. The values varied less than 30% for all steps.

<table>
<thead>
<tr>
<th>Step</th>
<th>Total activity (pmol)</th>
<th>Total protein (mg)</th>
<th>Specific activity (pmol/mg)</th>
<th>Yield (%)</th>
<th>Purification 34,000-Da protein</th>
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<td>Membranes</td>
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<td>8,700</td>
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<td>100</td>
<td>1</td>
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<td>0.418$^a$</td>
<td>1.10</td>
<td>12</td>
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<tr>
<td>Hydroxylapatite</td>
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<td>0.030$^a$</td>
<td>10,000</td>
<td>7.8</td>
<td>22,700</td>
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<td>Re-XAC-agarose</td>
<td>160</td>
<td>0.0073$^a$</td>
<td>21,900</td>
<td>4.2</td>
<td>49,800</td>
</tr>
</tbody>
</table>

$^a$ Binding activities were assessed at 5 nM [3H]DPCPX under the conditions described under “Experimental Procedures.”

Protein content was determined by the Amido Schwarz method (23).

Reaffinity XAC-agarose chromatography.

These results indicate that the purified $A_1$ receptor consists of an $M_r = 34,000$ polypeptide chain. Fig. 2 also shows the radioiodination pattern of the purified receptor protein (lanes E and F) to confirm the purity of the purified preparation.

Binding Properties of the Purified $A_1$ Adenosine Receptor—[3H]DPCPX binding to the various $A_1$ adenosine receptor preparations was determined by the polyethyleneimine filtration assay method as described under “Experimental Procedures.” Addition of the “pass-through fractions” to the assay mixture was found to restore the binding activity up to 2-4-fold for the highly purified receptor preparations such as the eluate from the affinity chromatography. No activation by the pass-through fractions was observed for the membrane or crude solubilized preparations. Dose-dependent experiments showed that very low concentrations of protein (10-15 ng/ml of assay mixture) were enough to show this effect (data not shown).

Time course experiments of [3H]DPCPX binding to the purified receptor preparations showed that the specific binding of [3H]DPCPX at 0 °C occurred relatively slowly and reached equilibrium after about 1 h. The binding was stable for at least 12 h at 0 °C. The dissociation of the bound [3H]DPCPX by the addition of an excess amount of XAC also occurred slowly with half-time of about 1 h ($k_1 = 0.013$ min$^{-1}$) (data not shown).

In saturation studies, the binding of [3H]DPCPX to the purified receptor was specific and saturable (Fig. 3). Parameters obtained from the linear Scatchard plot (Fig. 3, inset) were $K_d = 1.40 \pm 0.04$ nM and maximum specific binding value of 24.0 ± 3.0 pmol/mg of protein ($n = 3$). This maximum specific binding of the purified $A_1$ adenosine receptor, 24 nmol/mg, based on the amount of protein estimated by the Amido Schwarz method (23), is close to the theoretical value of 29.4 nmol/mg calculated on the basis of one binding site/34,000-Da protein. The $K_d$ value 1.40 nM is a little higher than the $K_d$ of 0.42 and 0.65 nM for [3H]DPCPX binding to intact rat brain membranes (26) and the digitonin-solubilized preparations (13), respectively. The specificity of the purified receptor was investigated by competition binding studies with various adenosine agonists and antagonists including adenosine as shown in Fig. 4. The rank order of potency for agonists and antagonists in competing for [3H]DPCPX binding agrees with the known specificity for the membrane-bound $A_1$ adenosine receptor (26, 27). Table II summarizes the affinities of adenosine ligands for the unpurified receptor (the extract with digitonin/cholate) and the purified receptor. The two preparations showed similar specificities for the adenosine ligands, but there were consistent differences in the affinities for agonists. The $K_i$ values for antagonist binding to the

![Diagram](image_url)

**FIG. 1.** Sequential chromatography steps during purification of the solubilized $A_1$ adenosine receptors from rat brain membranes. [3H]DPCPX-binding activity in each column determined with 4 nM [3H]DPCPX under the conditions described under “Experimental Procedures” is shown by (C). A and B, XAC-agarose chromatography. Solubilized membranes (1000 ml) were applied to a 70-ml column of XAC-agarose. The XAC-agarose column was then washed with buffer A and eluted with CPT solution. Fractions of 10 ml were collected and assayed for [3H]DPCPX binding. Recovery of [3H]DPCPX-binding activity and proteins at the various steps of the XAC-agarose chromatography expressed as a function of the total amount applied to the column is shown in A. The elution profile of [3H]DPCPX-binding activity from the XAC-agarose column is shown in B. The arrow indicates the beginning of the elution buffer. C, hydroxylapatite column chromatography. The active fractions (40 ml) from XAC-agarose chromatography were pooled and loaded onto the column, washed successively with buffer A (arrow 1), 10 mM (arrow 2), and 110 mM (arrow 3) potassium phosphate buffer containing 100 mM NaCl and 0.1% digitonin, and eluted with 500 mM potassium phosphate buffer containing 100 mM NaCl and 0.1% digitonin (arrow 4) at the positions indicated by the arrows. D, reaffinity XAC-agarose chromatography. The eluate from the hydroxylapatite column was loaded on a 4-ml column of XAC-agarose. The column was then washed with buffer A (arrow 1) and eluted with CPT solution (arrow 2) at the positions indicated by the arrows.
A purified receptor were essentially identical to the corresponding values for the unpurified receptor. On the other hand, the $K_I$ values for the agonists binding to the purified receptor were 17-fold higher than the corresponding values for the unpurified receptor.

$[^3H]DPCP$X-binding activity of the purified receptor preparation was found to be relatively stable. More than 80% of the original binding activity was preserved after the purified receptor preparations were stored at $-85^\circ$C.

**DISCUSSION**

In this work, the purification of the $A_1$ adenosine receptor from rat brain membranes to apparent homogeneity has been described. The purification scheme involved the use of an effective affinity chromatography step, XAC-agarose, followed by hydroxylapatite chromatography, and finally reactivity chromatography on XAC-agarose. This procedure resulted in a receptor preparation that has a specific activity of approximately 24 nmoles of $[^3H]DPCP$X binding/mg of protein and displays a single band of $M_r$ $\sim$ 34,000 in SDS gels after either radiodination or protein staining. The $M_r$ $\sim$ 34,000 band was also specifically labeled with $[^3H]$dipyridamole affinity-labeling agent, $[^3H]p$-DITC-XAC, showing that this peptide contains the ligand-binding site of the $A_1$ adenosine receptor. The purified preparations also displayed the expected ligand-binding specificity for various adenosine agonists and antagonists.

Attempts to purify $A_1$ adenosine receptors have met with little success previously because of the difficulty in obtaining active soluble receptors in a high yield and the lack of specific affinity resins. Although $A_1$ adenosine receptors have been solubilized from brains with various detergents under different conditions (8–11), a more consistent solubilization method was necessary to obtain a large amount of active soluble receptors. The condition for the solubilization used in this study gave consistently 30-40% yield of solubilization of $A_1$ adenosine receptors from rat brain membranes. It should be noted that addition of low concentrations of NaCl and cholate in the solubilization buffer and keeping the ratio of volumes of membrane pellets to solubilization buffer to be 1:10-1:13 are important to obtain a consistent yield of the solubilization.

Recently, a biospecific affinity chromatography system using XAC-immobilized agarose for the partial purification of $A_1$ adenosine receptors from rat brain membranes has been developed (12, 13). However, only a modest purification (150-fold) was achieved at that time because a significant amount of binding activity was lost during the affinity chromatography if the affinity column had been washed extensively with buffer before the specific elution of the receptor with CPT. In this study, it was found that the low yield of $[^3H]DPCP$X-binding activity from the affinity column could be restored by adding a small amount of proteins (<15 $\mu$g/ml) derived from the pass-through fractions in the first affinity chromatography. Addition of these pass-through fractions into the assay A containing 5 mM MgCl$_2$ at the protein concentration of 0.04-1 $\mu$g/ml. The purified receptor was also relatively stable when stored at $-85^\circ$C.

**Gel Permeation Chromatography of Purified $A_1$ Adenosine Receptor**—In order to study the hydrodynamic properties of the receptor and also to confirm that $[^3H]DPCP$X-binding activity resided on the $M_r$ $\sim$ 34,000 protein, the purified preparation from the second affinity chromatography was chromatographed on a gel permeation column (TSK-3000 SW). Fractions were assayed for $[^3H]DPCP$X binding, and the remaining portion was lyophilized and subjected to SDS-PAGE. As shown in Fig. 5, $[^3H]DPCP$X-binding activity was eluted as a single peak with apparent $M_r$ $= 150,000$ and Stokes radius of 5.0 nm. It was also shown that the appearance of the 34,000-Da peptide in the SDS-PAGE coincided with that of the $[^3H]DPCP$X-binding activity following gel permeation chromatography. Unpurified solubilized preparations (extract with digitonin/cholate) were also run in the same gel permeation column. The specific $[^3H]DPCP$X-binding activity was eluted in a position that corresponds to apparent molecular size of 288,000 and Stokes radius of 6.1 nm (data not shown).

**Fig. 2. SDS-PAGE analyses of adenosine receptors.** A, SDS-PAGE of adenosine $A_1$ receptor preparations at various stages of the purification stained by silver. Lane $A$, the first XAC-agarose chromatography eluate; lane $B$, hydroxylapatite eluate; lane $C$, the second XAC-agarose eluate; lane $D$, the same as lane $C$ except that SDS-PAGE was performed under nonreducing conditions. Approximately 100 ng of protein was applied to each lane. $B$, SDS-PAGE of purified $A_1$ adenosine receptors after several treatments. Lanes $A$–$D$, purified $A_1$ receptors labeled with $[^3H]p$-DITC-XAC in the presence of 1 $\mu$M XAC (lanes $A$ and $C$) or in the absence of XAC (lanes $B$ and $D$). Lanes $A$ and $B$ were silver stained, and lanes $C$ and $D$ were autoradiograms. Approximately 70 ng of protein was loaded on each lane. Lanes $E$ and $F$, autoradiogram of $[^3H]$labeled purified $A_1$ adenosine receptors by the chloramine-T method. Approximately 5,000 (lane $F$) and 10,000 cpm (lane $E$) of the iodinated receptors were loaded on the gels.
mixture increased the [³H]DPCPX binding of the highly purified receptor preparations to 2-4-fold. However, almost the same high [³H]DPCPX binding was obtained even without the pass-through fractions when the gel filtration method (15) was employed to separate free ligand from ligand-bound receptor instead of filtration with polyethyleneimine-treated filters (16). These results suggest that the pass-through fractions stimulate the ability of the polyethyleneimine-treated filters to adsorb very small amounts of [³H]DPCPX-bound receptor proteins or prevent the loss of very small amounts of [³H]DPCPX-bound receptor proteins during the transfer from the incubation tube to the filtration apparatus, although the precise mechanism was not known. Because there were not significant differences in the observed [³H]DPCPX-binding activity between these two assay methods as long as the pass-through fractions were added in the assay mixture, the...
**Table II**

Affinities of digitonin-solubilized and purified A<sub>1</sub> adenosine receptors for adenosine agonists and antagonists

For each adenosine ligand, competitive inhibition experiments were performed as described in the legend to Fig. 4. IC<sub>50</sub> and Hill coefficient values were derived from nonlinear curve fitting to a logistic model (25) using a computer program, Graph PAD. The IC<sub>50</sub> values were converted to K<sub>i</sub> values by the Cheng-Prusoff equation. The values listed in this table are means of two separate determinations. Abbreviations used are: PIA, N<sup>6</sup>-phenylisopropyladenosine; NECA, 5'-N-ethylcarboxamidoadenosine; 8PT, 8-phenyltheophylline; IBMX, 3-isobutyl-1-methylxanthine; ND, not determined.

<table>
<thead>
<tr>
<th>Ligand</th>
<th>Solubilized K&lt;sub&gt;i&lt;/sub&gt; (nM)</th>
<th>Hill coefficient</th>
<th>Purified K&lt;sub&gt;i&lt;/sub&gt; (nM)</th>
<th>Hill coefficient</th>
</tr>
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<tbody>
<tr>
<td><strong>Agonists</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(R)-PIA</td>
<td>1.3</td>
<td>0.77</td>
<td>23</td>
<td>0.82</td>
</tr>
<tr>
<td>Adenosine&lt;sup&gt;a&lt;/sup&gt;</td>
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<td></td>
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<tr>
<td>NECA</td>
<td>7.0</td>
<td>0.72</td>
<td>124</td>
<td>0.91</td>
</tr>
<tr>
<td>(S)-PIA</td>
<td>59</td>
<td>0.58</td>
<td>950</td>
<td>0.75</td>
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<tr>
<td><strong>Antagonists</strong></td>
<td></td>
<td></td>
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<tr>
<td>DPCPX</td>
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</tr>
<tr>
<td>CPT</td>
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<tr>
<td>IBMX</td>
<td>2400</td>
<td>0.95</td>
<td>5600</td>
<td>1.1</td>
</tr>
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</table>

<sup>a</sup> For the competition by adenosine, the receptor-binding assays were performed without the addition of the pass-through fractions in order to prevent the enzymatic degradation of adenosine during the incubation. The K<sub>i</sub> value for the purified A<sub>1</sub> adenosine receptor in that condition was estimated to be approximately 2.7 nM (data not shown).

polyethyleneimine-treated filter method was employed throughout this study.

An approximately 50,000-fold purification of the receptor from crude membranes using the purification method described above has been achieved. The maximum binding activity, 24 nmol/mg of protein, is consistent with the theoretical specific binding value of 29.4 nmol/mg of protein on the basis of the assumption that the ligand-binding subunit of the receptor resides on a peptide of M<sub>r</sub> ~ 34,000.

The purified A<sub>1</sub> adenosine receptor migrated on SDS-PAGE with M<sub>r</sub> = 34,000 either in the absence or presence of 2-mercaptoethanol, suggesting that the receptor does not contain disulfide-linked subunits (Fig. 2A). This molecular weight agrees with the reported values (M<sub>r</sub> = 35,000–38,000) for the labeled peptides obtained by the photoaffinity-labeling experiments using brain membranes as the receptor source (3–5). Identification of the M<sub>r</sub> ~ 34,000 protein was performed by affinity labeling using a specific acylating reagent, [3H]p-DITC-XAC, for A<sub>1</sub> adenosine receptor and also by gel permeation chromatography. It has been shown previously that [3H]p-DITC-XAC can be incorporated into the A<sub>1</sub> adenosine receptor protein of rat or bovine brain membranes (17). As shown in Fig. 2B, the alkylation of the purified A<sub>1</sub> adenosine receptor with this reagent was successful and could be blocked with XAC. Gel permeation chromatography of the purified preparation (Fig. 5) showed that the elution pattern of [3H]DPCPX-binding activity coincided with the intensity of the M<sub>r</sub> ~ 34,000 band in SDS-PAGE gels, suggesting again that the M<sub>r</sub> ~ 34,000 peptide is the A<sub>1</sub> receptor protein. It is of interest that the apparent molecular size of the purified A<sub>1</sub> adenosine receptor was significantly smaller than that of the unpurified receptor preparation determined by gel permeation chromatography. These results may indicate that receptor-associated proteins (such as G<sub>i</sub>) had been dissociated in the purified preparation. It has been observed that activation effects of guanine nucleotides on [3H]DPCPX binding to solubilized A<sub>1</sub> adenosine receptors disappeared after the purification with XAC affinity chromatography (13). The apparent molecular weight of 150,000 for the purified receptor estimated from the gel permeation chromatography is significantly larger than either the value of 34,000 determined with SDS-PAGE or the value of 63,000 estimated from a target size analysis described below. These results suggest that the purified receptor exists as a polymer, although high contents of bound detergents in the receptor molecule can also explain these results. More detailed size exclusion chromatography experiments in combination with sucrose density centrifugation and direct determination of amounts of bound detergents are necessary to determine the accurate molecular size of the receptor.

Because the receptor protein size of 35,000–38,000 Da obtained from the photoaffinity labeling (3–5) is somewhat smaller than might have been anticipated for a receptor that is coupled to adenylate cyclase such as adrenergic receptors (58,000–63,000) and muscarinic acetylcholine receptors (70,000) (28–29), it has been questioned whether the photo-labeled adenosine target protein is the real minimum unit of the A<sub>1</sub> adenosine receptor or one subunit of the more complex receptor assembly or a small labeled fragment produced by the UV irradiation. The results with SDS-PAGE in this study clearly demonstrated that the M<sub>r</sub> ~ 34,000 peptide is the...
minimum unit of the \( A_1 \) adenosine receptor itself, and no larger subunits of the receptor exist, although it is still to be determined whether the \( A_1 \) adenosine receptor consists of a monomer or polymer of \( M_e = 34,000 \) peptide. Frame et al. (30) reported that the \( M_e \) of \( A_1 \) adenosine receptor in intact brain membranes is approximately 63,000 from the radiation inactivation experiments, suggesting a subunit structure of this receptor in combination with the data of the affinity labeling.

The \( M_e = 34,000 \) peptide band in SDS-PAGE of the purified \( A_1 \) adenosine receptor was always broader than the bands of marker proteins irrespective of the detection methods. A similar broadness has been observed for the purified receptors including stereospecificity and agonist or antagonist rank affinity states, including the affinity labeling. The affinity of adenosine with purified \( A_1 \) adenosine receptors was directly estimated in this study. Adenosine showed the affinity of adenosine with unpurified \( A_1 \) adenosine receptor is assumed as an intact adenosine receptor. The molecular weight of the receptor remain intact during the purification procedure, i.e. high affinity state for agonists can also be explained by the dissociation of G proteins, which are important for agonists binding from \( A_1 \) receptors during the purification. In addition, the affinity of adenosine with purified \( A_1 \) adenosine receptors was directly estimated in this study. Adenosine showed the similar affinity (\( K_I = 100 \) nM) with the purified receptors as 5'-N-ethycarbaminoadenosine showed. The \( K_I \) value for adenosine with unpurified \( A_1 \) adenosine receptor is assumed to be \( \sim 6 \) nM using the same ratio of \( K_I \) values between unpurified and purified preparations for other agonists, although adenosine has been considered to be a poor ligand for either \( A_1 \) or \( A_2 \) adenosine receptors in brain tissue (37).

In summary, these studies present the first purification of the \( A_1 \) adenosine receptor, which has appropriate properties as an intact adenosine receptor. The molecular weight of the receptor is \( \sim 34,000 \) from SDS-PAGE. These findings should facilitate the molecular characterization of the receptor including sequencing of the receptor, cloning of the gene, and reconstitution studies with individual G proteins as well as the production of specific antireceptor antibodies.

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REFERENCES