

Active opioid binding protein from rat brain

— a glycoprotein containing α -methyl-D-mannoside residues¹

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ABSTRACT Active opioid binding protein was partially purified from rat brain by *Vicia bungei* Ohwi lectin (VBL). Mannose also existed in opioid receptors since the binding between opioid binding protein and VBL is specific, via α -methyl-D-mannoside (MeMan) residues. Since the active opioid binding protein purified by VBL chromatography was enriched about 200-folds. VBL can be used as effective purification tool which is much better than wheat germ agglutinin (WGA), a lectin commonly used in purifying opioid receptor.

KEY WORDS endorphin receptors; carrier proteins; lectins

The glycoprotein nature of opioid receptor has been elucidated⁽¹⁾. However, little is known about the carbohydrate composition of opioid receptor except that they contain *N*-acetylglucosamine (GlcNAc) which was deduced from the wheat germ agglutinin (WGA) binding of the receptor. We have successfully solubilized the active opioid receptor from rat brain using digitonin, and verified the fact that our solubilized preparation possesses the characteristics similar to those of the membrane bound receptor⁽²⁾. Herewith, we report that mannose also exists in opioid receptor since there is specific binding between opioid binding protein and *Vicia bungei* Ohwi lectin (VBL), via α -methyl-D-mannoside

(MeMan) residues. Meanwhile, we found that VBL can be used as an effective purification tool which is much better than WGA, a lectin commonly used in purifying opioid receptor. In fact, we have got apparently homogeneous opioid binding protein by using VBL-sepharose and 6-succinyl-morphine - (CH₂)₆-sepharose chromatography⁽³⁾.

MATERIALS AND METHODS

[³H]Diprenorphin (444 GBq/mmol, Amersham Co). [³H]Etorphine (1.48 TBq/mmol), unlabeled etorphine and naloxone were got from Shanghai Medical University. VBL was isolated and purified by affinity chromatography on porcine thyroglobulin-ABSE-crosslinked agar⁽⁴⁾. The sugar hapten of this lectin has been proved to be MeMan. WGA-agarose (Vector Lab Inc). Sepharose 4 B (Pharmacia), MeMan (Fluka). Digitonin (Hangzhou First Pharmaceutical Factory), 1300-100 adsorbent resin (Shanghai Institute of Pharmaceutical Industry).

RESULTS

Solubilization of opioid receptor from rat brain By the method previously used in our laboratory^(3,5), crude rat brain membrane fraction (P₂) and the solubilized opioid receptor preparation were prepared. Briefly, 1% of digitonin and 5 mmol/L of MgSO₄ were added to P₂ fraction. After thorough shaking at 4°C for 20 min

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centrifugation at $100\,000 \times g$ for 60 min was followed. The 13X0-100 adsorbent resins were added to the supernatant at the ratio of 0.5 g/ml and shaken at 4°C for 60 min to absorb the excess detergent.

Precipitation test of solubilized opioid receptor with lectin The precipitation test for binding of lectin and membrane receptor was first used in studying acetylcholine receptor⁽⁶⁾ and was modified by us in opioid receptor research⁽²⁾. In brief, VBL 0.5 mg/ml was incubated with about 1-3 mg solubilized opioid receptor in the presence or absence of MeMan 0.2 mol/L at 8°C for 4 h, then centrifuged at $10\,000 \times g$ for binding assay. In the tubes without sugar, the specific binding with [^3H]diprenorphin (4 nmol/L) lost completely as opioid receptor protein combined with VBL, then precipitated. But in case when MeMan (0.2 mol/L) existed in excess, opioid receptor mostly bound to sugar rather than VBL, so neither solubilized receptor precipitated nor the specific binding reduced in the supernatant (Tab 1). This experiment indicated that opioid receptor from rat brain contained MeMan residues suitable for VBL binding.

Partial purification of opioid receptor by VBL-sepharose VBL was coupled to sepharose 4B by CNBr method⁽⁷⁾. In the typical experiment, 5 ml of solubilized preparation was applied to 10 ml of the column at the fluid rate of 8 ml/h. The column was pre-equilibrated and flushed with 0.02% of digitonin, 5 mmol/L of MgSO_4

in Tris-HCl (50 mmol/L), pH 7.5 (Buffer A) until the absorbance of protein at 206 nm was near zero. Opioid receptor was then eluted from the column with 5 ml of MeMan 0.1 mol/L in Buffer A. Fractions were collected to measure the opioid binding activity. Results showed that opioid binding protein was enriched to 170-190 folds by VBL-sepharose 4B chromatography. Saturation curve and Scatchard analysis for the binding of [^3H]etorphine to the partially purified opioid receptor is shown in Fig 1. Meanwhile, we have checked the WGA column under the same experimental conditions except using 0.1 mmol/L of GlcNAc in Buffer A. Obviously, VBL was much better than WGA in purifying opioid receptor (Tab 1).

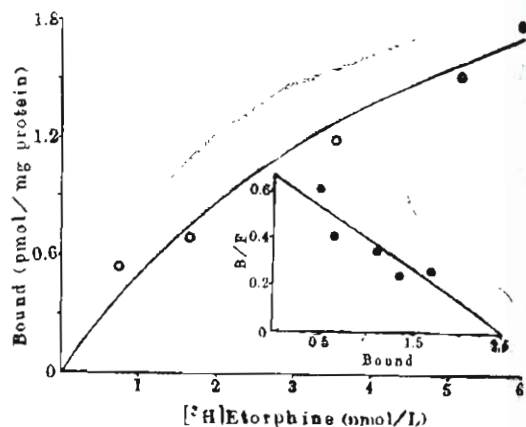


Fig 1. Saturation curve and Scatchard analysis of [^3H]etorphine binding to opioid receptor partially purified by *Vicia bungei* Ohwi lectin. $n=3$. $K_D=4.4 \pm 0.9$ nmol/L; $B_{max}=3.2 \pm 0.8$ pmol/mg protein

Tab 1. Purification efficacy by *Vicia bungei* Ohwi lectin (VBL) and wheat germ agglutinin (WGA) affinity chromatography. [^3H]Etorphine 4 nmol/L were used in binding assays.

Elution from	Expts	Specific binding (fmol/mg protein)	Purification fold
Control	3	30.5; 27.4; 24.4	—
VBL	3	4658; 5150; 5642	170-188
WGA	2	95.4; 191.8	4-7

In case of SDS-polyacrylamide gel electrophoresis, there were 5-7 bands in elution of VBL column with the major band of Mr. 45 000 which had opioid binding activity, and many more bands in WGA column elution. Polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate (SDS) was performed on 1.0-1.5 mm thick slab gels by using 9.3% separating gel and 4.2% stacking gel⁽⁸⁾.

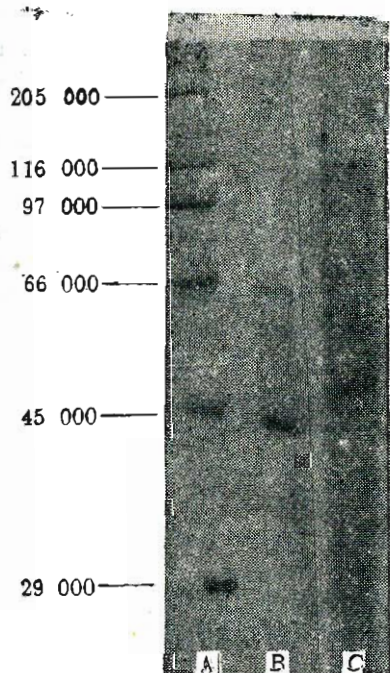


Fig 2. SDS-PAGE analysis of the elutions from VBL-sepharose and WGA-agarose. A) molecular weight markers (carbonic anhydrase, albumin from egg, albumin from bovine, phosphorylase b, β -galactosidase and myosin); B) MeMan elution of VBL column; C) *N*-acetyl-glucosamine elution of WGA column.

Protein bands were stained by 0.1% Coomassie blue (Fig 2).

Opioid receptor binding assay was performed according to the polyethylene glycol precipitation method⁽⁹⁾. Protein concentration was determined by the Coomassie blue dye stain method⁽¹⁰⁾.

DISCUSSION

Gioannini *et al*⁽¹⁾ have shown the presence of sugar residues by their retention on the immobilized lectin WGA. We have confirmed their finding that there are GlcNAc residues in opioid receptor by precipitation method⁽²⁾. We have studied the interaction between WGA, VBL and the opioid receptors, by using the method of precipitation of solubilized receptors by lectins. Our experiment gives a strong support to

the view of glycoprotein nature of opioid receptors, i. e. besides WGA, VBL had also high affinity with receptors, by using the precipitation method⁽²⁾. Now we found that digitonin-solubilized opioid receptor from rat brain has also MeMan residues as proved by both precipitation method and VBL chromatography. It is interesting that some other lectins with MeMan hapten, such as concanavalin A (Con A) and pisum lectin (PSL), do not show any binding activity with opioid receptor⁽²⁾. As we know, some lectins are quite delicate about even minute structural change at special position of sugar hapten. We assume that there may be some group replacement in C_4 and C_6 position of MeMan in opioid receptor, VBL can recognize and have binding activity with it but Con A and PSL can not.

Many laboratories have tried to use WGA column to purify opioid receptor and so did we. But the result was not satisfactory. Our data show that WGA has much less affinity with opioid receptor than VBL. From WGA column, we can only enrich receptor less than 10-folds, but by using VBL column, the purification can achieve about 200-folds. So we believe that VBL is a new purification tool in opioid receptor research field.

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大鼠脑活性阿片结合蛋白 —— 一种含有甘露糖基的糖蛋白¹

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摘要 大鼠脑活性阿片结合蛋白借助三齿草藤凝集素(VBL)获得部分纯化。活性阿片结合蛋白由 α -甲基-D-甘露糖基显示与VBL的特异性结合, 证实阿片受体也含有甘露糖基, 活性阿片结合蛋白经VBL柱纯

化约200倍。VBL可以用作阿片受体的纯化工具, 它比常用的受体纯化试剂麦胚凝集素(WGA)更好。

关键词 内啡肽受体; 载体蛋白; 凝集素

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兔脑室注射山莨菪碱引起发作性放电的作用部位及安定的拮抗作用¹

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Sites of seizure discharges after intracerebroventricular injection of anisodamine and the antagonism by diazepam in rabbits¹

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ABSTRACT Anisodamine is a tropine alkaloid isolated from *Scopolia tangutica*

Maxim. To determine the original sites of anisodamine seizure discharge, permanent electrodes were implanted into different parts of the brain in rabbits and the electrical activities were continuously recorded by monopolar leads. Injection of anisodamine 1.5 mg/kg into the lateral ventricle of conscious rabbits always produced abnormal discharges. The spike discharges

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