The Oligosaccharide Moiety of the β_1-Adrenergic Receptor from Turkey Erythrocytes Has a Biantennary, *N*-Acetyllactosamine-Containing Structure†

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ABSTRACT: The turkey erythrocyte β_1-adrenergic receptor can be purified by affinity chromatography on alprenolol–Sepharose and characterized by photoaffinity labeling with *N*-(*p*-azido-*m*-[^{125}I]iodobenzyl)-carazolol. Through the use of the specific glycosidases neuraminidase and endo-β-*N*-acetylglucosaminidase H and affinity chromatography on lectin–Sepharose gels, we show here that the receptor is an *N*-glycosyl protein that contains complex carbohydrate chains. No high-mannose carbohydrate chains appear to be present. The binding of the radiolabeled antagonist dihydroalprenolol to the receptor is affected neither by the enzymic treatments nor by the presence of lectins, suggesting that the carbohydrate moiety is not involved in the catecholamine binding site.

The glycoprotein nature of proteins has generally been characterized by using the carbohydrate binding properties of lectins that interact specifically not only with free or terminal monosaccharides but also with oligosaccharidic sequences. The affinity of lectins for free sugars is modified by the nature of the linkage between terminal sugars and other residues (Kornfeld et al., 1975; Debray et al., 1981). Sequential procedures using various lectins allow tentative definitions of saccharidic sequences. For example, glycoproteins with terminal neuraminic acid are retained on limulin–Sepharose affinity gels. Treatment of these proteins with neuraminidase may unmask new terminal saccharides that become accessible to binding by other lectins.

Many plasma membrane proteins are glycosylated, and receptors are no exceptions: several receptors have been described as being glycoproteins, including those for opiates (Gioannini et al., 1982), insulin (Cuatrecasas, 1973a; Hedo et al., 1981; Cherqui et al., 1981; Harrison & Itin, 1980), nerve growth factor (Buxser et al., 1983), muscarinic ligands (Shirakawa et al., 1983), and epidermal growth factor (Carpenter, 1977) and most recently mammalian β_2-adrenergic receptor (Stiles et al., 1984).

The structure and the function of the β_1-adrenergic receptor from turkey erythrocyte plasma membrane have been analyzed by various methods including affinity chromatography (Vauquelin et al., 1977, 1979a) and immunological methods (Couraud et al., 1981; Strosberg et al., 1982). It has been shown that the receptor contains one or several disulfide bonds (Vauquelin et al., 1979b) essential for ligand binding activity and that a thiol group may be modified by *N*-ethylmaleimide in the presence of only agonist, not antagonist (Bottari et al., 1979; Vauquelin et al., 1980).

This paper describes the results of our investigations on the β_1-adrenergic receptor from turkey erythrocytes. Using six different lectins, we followed the (−)-[^{3}H]dihydroalprenolol ([^{3}H]DHA)[1] binding activity of the receptor pretreated or not with neuraminidase or Endo H, and we found that the turkey erythrocyte receptor is indeed a glycoprotein with a complex carbohydrate chain linked to the polypeptide chain via the amide group of an asparaginyl residue. Since neither the binding of the lectins nor the treatment with neuraminidase or endo-β-*N*-glucosaminidase H affects the interaction with the β-adrenergic ligands, it is likely that the carbohydrate moiety is not close to the receptor binding site.

Experimental Procedures

Materials. The lectins used in this work are listed in Table I, with their carbohydrate specificity. WGA,[1] PNA, and Con A were purchased from Boehringer, LPA was from Sigma, RCA_I–Sepharose was from Miles-Yeda Ltd., UEA-F–Sepharose was from E-Y Laboratories, and PNA–Ultrogel and Con A–Sepharose were from Pharmacia. WGA– and LPA–Sepharose were prepared according to the instructions supplied by the manufacturer Pharmacia. (−)-Propranolol and (+)-propranolol were purchased from ICI and (−)-epinephrine, (−)-norepinephrine, and (−)- and (+)-isoproterenol from Sigma; (±)-alprenolol was the generous gift of Ciba-Geigy. [^{3}H]Dihydroalprenolol (40 Ci/mmol) was supplied by New England Nuclear, and neuraminidase from *Clostridium perfringens* (EC 3.2.1.18) (0.6 unit/mg) and endo-β-*N*-acetylglucosaminidase H from *Streptomyces griseus* (30 units/mg of enzyme) were purchased from Boehringer. D-Galactose and *N*-acetyl-D-glucosamine were obtained from Sigma; L-fucose was obtained from Aldrich and methyl α-D-mannoside from Calbiochem.

Membrane Preparation and Solubilization. Turkey erythrocyte plasma membranes were prepared and solubilized with 0.25% digitonin as described previously (Durieu-Traut-

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[1] Abbreviations: EDTA, ethylenediaminetetraacetic acid; [^{3}H]DHA, (−)-[^{3}H]dihydroalprenolol; PMSF, phenylmethanesulfonyl fluoride; NeuAc, *N*-acetylneuraminic acid; Gal, D-galactose; GlcNAc, *N*-acetyl-D-glucosamine; Man, D-mannose; Fuc, L-fucose; Asn, L-asparagine; EGF, epidermal growth factor; NGF, nerve growth factor; Con A, concanavalin A; LPA, *Limulus polyphemus* agglutinin; PNA, peanut agglutinin; RCA_I, *Ricinus communis* agglutinin I; UEA-F, *Ulex europaeus* agglutinin; WGA, wheat germ agglutinin; Endo H, endo-β-*N*-acetylglucosaminidase H; SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis; Tris-HCl, tris(hydroxymethyl)aminomethane hydrochloride; KIE, kallikrein inhibitor.

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Table I: Specificities of Lectins Used in This Study

lectins	sugars	saccharidic sequences	references
concanavalin A (Con A)	terminal or internal α-D-mannose	biantennary glycopeptide oligomannoside	Debray et al. (1981), Narasimhan et al. (1978), Debray et al. (1983)
Limulus polyphemus (LPA)	terminal *N*-acetyl- or *N*-glycolylneuraminic acid	*N,N',N''*-triacetylchitotriose	Roche et al. (1974)
peanut agglutinin (PNA)	terminal D-galactose	galactosyl-(β-1,3)-*N*-acetyl-D-galactosamine	Lotan et al. (1975), Pereira et al. (1976)
Ricinus communis agglutinin (RCA_I)	terminal or internal D-galactose	galactosyl-(β-1,4)-*N*-acetylglucosamine	Debray et al. (1981), Nicolson et al. (1974), Debray et al. (1983)
Ulex europaeus agglutinin (UEA-F)	terminal α-L-fucose	L-fucosyl-(α-1,6)-*N*-acetylglucosamine	Debray et al. (1981), Kornfeld et al. (1981), Debray et al. (1983)
wheat germ agglutinin (WGA)	terminal or internal *N*-acetylglucosamine and neuraminic acid	*N*-acetylglucosaminyl-(β-1,4)-*N*-acetylglucosaminyl-(β-1,*N*)-asparagine	Debray et al. (1981), Nagata et al. (1974), Peters et al. (1979), Debray et al. (1983)

mann et al., 1980), except that bacitracin (0.1 mg/mL) and protease inhibitors (5 mM EDTA, 1 mM PMSF, 100 milliunits of KIE trasylol) were added to all buffers.

The characteristics of the binding of [^{3}H]DHA to membranes and to solubilized receptor prepared in the presence of EDTA were the same as those of membranes prepared without EDTA.

Binding Assays. The same procedure was used to determine the binding of [^{3}H]DHA to the solubilized receptor and to the fractions from lectin columns (Vauquelin et al., 1977). Specific binding was defined as the difference between the binding of [^{3}H]DHA in the presence and in the absence of 50 μM of (±)-alprenolol. The sugars used for elution had no effect on the [^{3}H]DHA binding.

When the effect of lectins on the binding of [^{3}H]DHA was tested, the solubilized receptor (0.2–0.4 mg of protein/mL) was preincubated for 60 min at 4 or 30 °C with concentrations of lectin varying from 25 to 500 μg/mL. Specific binding was then measured as described above.

Lectin Affinity Chromatography. Lectin columns with bed volumes of 1 mL were washed before use with at least 30 mL of 10 mM Tris-HCl (pH 7.4), 5 mM EDTA, 90 mM NaCl, 0.05% (w/v) digitonin, and 1 mM PMSF (buffer A). Solubilized preparations (2–3 mL) containing 1–3 mg of protein were loaded on the lectin column and recycled 3 times through the column. Unbound material was collected in the flow-through (fraction 1). The column was washed with 10 mL of buffer A and then with 10 mL of 10 mM Tris-HCl (pH 7.4), 5 mM EDTA, 200 mM NaCl, 0.05% (w/v) digitonin, and 1 mM PMSF (buffer B) (fraction 2). These steps were performed at room temperature.

Bound material was eluted by addition of 300 mM specific sugar to buffer B. The flow of the column was 100 μL/min, and five fractions of 1 mL were collected (fractions 3–7). Elution was performed at 4 °C.

In order to control the specificity of the binding on the lectin column, the specific sugar was added to the solubilized receptor before chromatography and the solubilized receptor was loaded on columns of Ultrogel or Sepharose free of lectin or substituted with immunoglobulin.

Alprenolol–Sepharose Affinity Chromatography. Alprenolol–Sepharose affinity chromatography of the digitonin-solubilized receptor was performed as described previously (Vauquelin et al., 1977, 1979a; Durieu-Trautmann et al., 1980). A column with 1 mL of alprenolol–Sepharose gel was equilibrated in buffer A and loaded with 6 mL of digitonin extract containing 5 mg of protein. The column was rapidly washed with 4 volumes of buffer A and then with 2 volumes of 10 mM Tris-HCl (pH 7.4), 5 mM EDTA, 1 M NaCl, 0.05% (w/v) digitonin, and 1 mM PMSF (buffer C). Elution of the receptor was achieved with 7 mL of buffer C containing 400 nM [^{3}H]DHA, at a flow rate of 100 μL/min. All the steps were performed at room temperature. Six milliliters of the eluted fraction was then loaded on a WGA column as described in the preceding paragraph.

In these experiments, the presence of the receptor was detected by binding of [^{3}H]DHA by use of the gel filtration technique (Vauquelin et al., 1979a).

Neuraminidase Digestion. Membranes were routinely incubated for 30 min at 30 °C with neuraminidase (from *Clostridium perfringens*) at a concentration of 5 milliunits/mg of protein in 1 mL of 10 mM Tris-HCl (pH 6.5), 5 mM EDTA, 1 mM PMSF, and 90 mM NaCl. At the end of the incubation, membranes were centrifuged for 10 min at 40000*g* and suspended in buffer A before solubilization. The neuraminic acid released into the medium was determined in the supernatant by the procedure of Warren (1959). Under these conditions 70% of the total neuraminic acid was released, i.e., 28 μg/mg of protein. The same results were obtained when membranes were preincubated with various concentrations of neuraminidase, from 3.5 to 13 milliunits/mg. Neuraminidase treatment had no effect on the [^{3}H]DHA binding.

Endo H Treatment. Membranes (30 mg) were incubated for 90 min at 30 °C with endo-β-*N*-acetylglucosaminidase H at a concentration of 1 milliunit/mg of protein in 5 mL of 10 mM Tris-HCl (pH 6.5), 5 mM EDTA, 1 mM PSMF, and 90 mM NaCl. At the end of the incubation, membranes were centrifuged and solubilized as described above.

Protein. Protein determinations were performed by the method of Bradford (1976) using bovine serum albumin as the standard.

Radioiodination. The receptor eluted from WGA–Sepharose and from alprenolol–Sepharose was labeled with ^{125}I by the chloramine-T technique (Greenwood et al., 1973) and lyophilized, as already described (Durieu-Trautmann et al., 1980).

Electrophoresis. SDS–polyacrylamide gel electrophoresis on slab gels was performed according to the method of Laemmli & Favre (1973) with a 4% stacking gel and a 10% separating gel. The lyophilized receptor was solubilized in sample buffer. The gel was dried and exposed on a Fuji film. The molecular weight markers used were carbonic anhydrase (M_r 31 000), serum albumin (M_r 69 000), and phosphorylase (M_r 97 000).

RESULTS

Chromatography of the β-Adrenergic Receptor on Lectin Columns. The turkey erythrocyte β-adrenergic receptor solubilized in 0.25% digitonin was chromatographed on six different immobilized lectins (Table I). As shown in Table

Table II: Recovery of Solubilized β-Adrenergic Receptor from Various Lectin Columns[a]

lectin column	sugar eluant (0.3 M)	recovery of specific (−)-[^{3}H]DHA binding (%) for fractions 1	2	4–6
wheat germ	*N*-acetylglucosamine	20 ± 5	<1	60 ± 3
concanavalin A	methyl α-D-mannoside	55 ± 3	20	14 ± 1
Limulus polyphemus	*N*-acetylneuraminic acid	72 ± 2	<1	10 ± 1
Ulex europaeus	fucose	50 ± 10	<1	42 ± 2
Ricinus communis	galactose	86 ± 5	<1	14 ± 2
peanut	galactose	70 ± 10	20	6 ± 2

[a] Lectin chromatography and [^{3}H]DHA binding were performed as described under Experimental Procedures. Fraction 1, flow-through; fraction 2, washes; fractions 4–6, eluates. The percentage of recovery was determined on the basis of total applied solubilized receptor. Each value represents the mean of two or three independent experiments.

Table III: Chromatography of Solubilized β-Adrenergic Receptor on Various Lectin Columns after Neuraminidase Treatment of the Membranes[a]

lectin columns	(−)-[^{3}H]DHA binding retained (%) untreated membranes	treated membranes
wheat germ	80 ± 5	60 ± 2
Limulus polyphemus	28 ± 2	0
Ricinus communis	20 ± 5	85 ± 5
peanut	30 ± 10	30 ± 5

[a] Lectin chromatography, [^{3}H]DHA binding, and neuraminidase treatment of the membranes were performed as described under Experimental Procedures. The percentage of binding was determined on the basis of total applied solubilized receptor. Each value represents the mean of two or three independent experiments.

II, Sepharose gels containing UEA-F lectin that binds fucose or WGA lectin that binds *N*-acetylglucosamine and to a lesser degree neuraminic acid retained a significant portion, 42% and 80%, respectively, of the [^{3}H]DHA binding; most or all the radioactivity was subsequently eluted by the appropriate sugar. The receptor was poorly adsorbed to PNA, Con A, RCA_I, and LPA lectins: 72–90% of the receptor was recovered in the flow-through and in the subsequent washes; 5–15% was eluted by the specific sugar.

The retained binding activity on Con A was eluted not only by mannose but also by *N*-acetylglucosamine. Retention was never observed when the specific sugar was included in the sample before chromatography.

These results show that the β-adrenergic receptor from turkey erythrocyte membranes is a glycoprotein containing at least fucose and *N*-acetylglucosamine and possibly neuraminic acid residues.

Lectin Chromatography of the β-Adrenergic Receptor after Neuraminidase Treatment of the Membranes. Treatment of membranes with neuraminidase (Table III), which removes neuraminic acid, completely suppressed the binding of the solubilized receptor on the LPA gel and thus confirmed that neuraminic acid residues were present on the carbohydrate moiety of the receptor. This result was corroborated by that obtained with WGA–Sepharose: the adsorption of the receptor on this gel was reduced from 80% to 60% after neuraminidase treatment, confirming the interaction of neuraminic acid residues with WGA.

The two lectins with specificity for galactose behaved very differently: PNA retained the same percentage of [^{3}H]DHA binding whether the membranes were pretreated or not by neuraminidase, while RCA_I, after such a treatment, retained

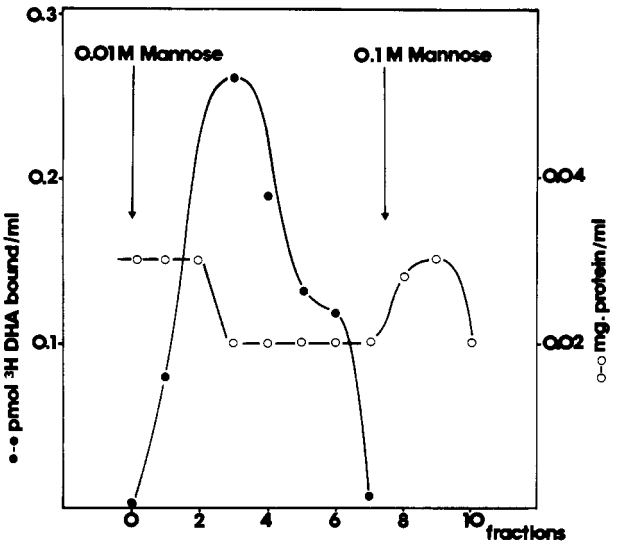


FIGURE 1: Affinity chromatography of solubilized membrane (2 mg) on a Con A column was performed as described under Experimental Procedures, except that elutions were done by 0.01 M mannose followed by 0.1 M mannose.

85% instead of 20% of the [^{3}H]DHA binding sites (Table III). This suggests that the receptor contained predominantly the structure galactosyl-(β-1,4)-*N*-acetylglucosamine (Table I) and that these residues were masked by neuraminic acid.

Con A Chromatography of the β-Adrenergic Receptor after Endo H Treatment of the Membranes. Treatment of membranes with Endo H, which removes the mannose residues from oligomannosidic glycopeptides (Borst et al., 1983), did not affect the retention of [^{3}H]DHA binding activity on Con A–agarose. However, the amount of retained total glycoprotein was decreased by 20%.

Thus it seems likely that the carbohydrate moiety of the β-receptor does not contain high-mannose chains; the decrease in the retention of proteins can be explained by the presence, in the membranes, of oligomannosidic glycopeptides not related to the β-adrenergic receptor.

Concanavalin A Chromatography Elution Profiles of the β-Adrenergic Receptor. To confirm the absence of oligomannoside structures on the β-receptor, differential elution (Narasimhan et al., 1978) of [^{3}H]DHA binding activity from the Con A column was performed. The elution pattern is shown in Figure 1. [^{3}H]DHA binding activity was eluted with 10 mM mannose, which indicates the relatively weak affinity of the β-glycoprotein receptor for the Con A gel, characteristic of the biantennary *N*-acetyllactosaminic-type glycopeptide. The fractions eluted with a higher concentration of mannose (100 mM) were devoid of [^{3}H]DHA binding activity.

Partial Purification of the β-Adrenergic Receptor by Lectin Chromatography. The [^{3}H]DHA binding capacities per milligram of protein of the lectin column's eluates were increased 5–22-fold (Table IV): PNA, RCA_I, LPA, and UEA-F were the least efficient (5–8-fold purification) with various recoveries (5–42%); Con A, whose highest specificity is for mannosides, allowed a 13-fold purification and a 13–15% recovery when retained receptors were eluted with mannose (Tables II and IV); optimum purification (22.5-fold) and recovery (60%) of the β-adrenergic receptor were obtained with WGA (Table II). Thus, the characteristics of the [^{3}H]DHA binding were examined in the eluate from the WGA gel and were found not to be altered: on the solubilized receptor (Figure 2) as on the WGA gel eluate (Figure 3) the [^{3}H]DHA binding was saturable and displaceable by β-adrenergic agonists and antagonists in a stereospecific way and with an

Table IV: Purification of Solubilized β-Adrenergic Receptor by Chromatography on Various Lectins[a]

	protein (mg)	(−)-[^{3}H]-DHA binding (pmol)	sp act. (pmol/mg of protein)	purification (x-fold)
solubilized receptor	1.7	2.05	1.2	1
wheat germ–Sepharose eluate	0.09	1.23	27.0	22.5
Con A–Sepharose eluate	0.016	0.27	16.0	13.3
peanut–Ultrogel eluate	0.02	0.14	7.0	5.8
Ricinus–Sepharose eluate	0.05	0.29	5.8	4.8
Ulex–Sepharose eluate	0.12	0.86	7.2	6.0
Limulus–Sepharose eluate	0.02	0.20	10	8.3

[a] Lectin chromatography, [^{3}H]DHA binding, and protein determinations were performed as described under Experimental Procedures. Purification referred to solubilized receptor and was estimated as the increase in binding capacity per milligram of protein.

order of potencies specific to the β_1-adrenergic receptor: (−)-isoproterenol > (−)-norepinephrine ≥ (−)-epinephrine (Tables V and VI). In both preparations, Scatchard analyses indicated that [^{3}H]DHA binds to a single class of noncooperative sites (Figures 1 and 2).

Lectin Chromatography of the β-Adrenergic Receptor Purified by Sepharose–Alprenolol Affinity Chromatography. To verify that the β-adrenergic receptor from turkey erythrocyte membrane is a glycoprotein, the receptor purified by alprenolol–Sepharose affinity chromatography as described earlier (Durieu-Trautmann et al., 1980) was applied to a WGA gel. The flow-through of the WGA column was found to contain a negligible amount (3%) of the [^{3}H]DHA binding activity, confirming the glycoproteic nature of the purified β-adrenergic receptor, which was almost completely (97%) retained on the WGA gel. The eluate from the two consecutive affinity gels and the eluate from the alprenolol–Sepharose gel (before its loading on the WGA column) were iodinated and submitted to SDS gel electrophoresis followed by autoradiography. In both cases two bands with apparent M_r values of 50 000 and 36 000 were revealed (Figure 4). Thus, the two polypeptides related to the purified receptor were glycosylated.

[^{3}H]DHA Binding in the Presence of Different Lectins. In an attempt to determine whether the sugars shown to be present on the β-adrenergic receptor were involved in the binding site of the hormone, solubilized receptor was prein-

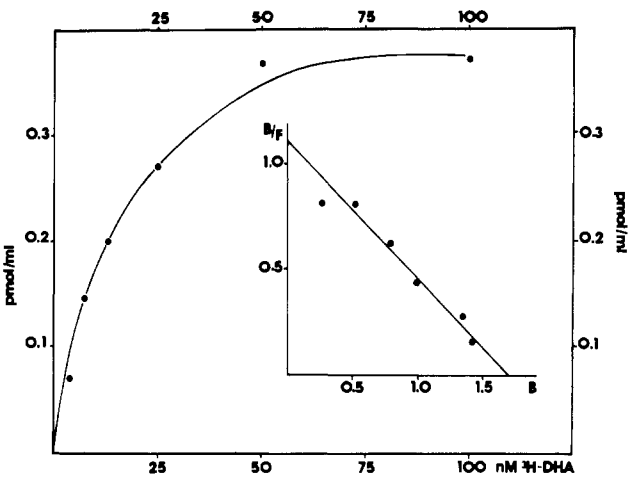


FIGURE 2: Binding of [^{3}H]DHA on solubilized turkey erythrocyte membranes. Aliquots of 600 μg of protein/mL were incubated with varying concentrations of radioligand (*F*), and specific binding was determined as described under Experimental Procedures. The inset shows a Scatchard analysis of the binding. *B* is the number of picomoles of radioligand specifically bound per milligram of protein.

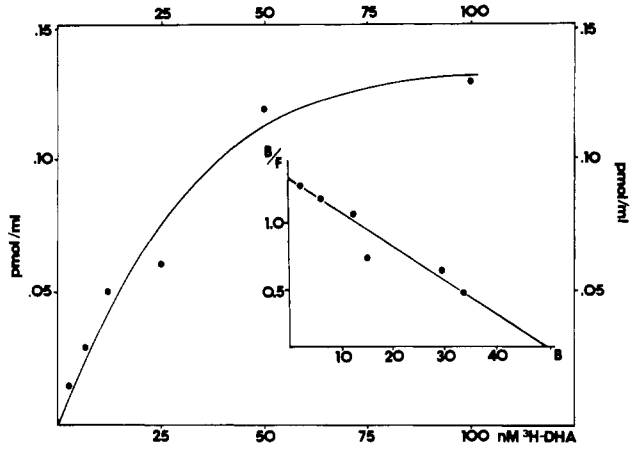


FIGURE 3: Binding of [^{3}H]DHA on eluate of WGA column. Aliquots of 4 μg of protein/mL were incubated with varying concentrations of radioligand (*F*), and specific binding was determined as described under Experimental Procedures. The inset shows a Scatchard analysis of the binding. *B* is the number of picomoles of radioligand specifically bound per milligram of protein.

Table V: Inhibition of [^{3}H]DHA Binding to Solubilized Membranes by Various Concentrations of (−) and (+) β-Adrenergic Ligands[a]

	[^{3}H]DHA binding (cpm/mL)					
ligand concn (M)	(−)-propranolol	(+)-propranolol	(−)-isoproterenol	(+)-isoproterenol	(−)-norepinephrine	(−)-epinephrine
10^{-4}	0	0	0	0	0	0
10^{-6}	203 ± 5	870 ± 20	377 ± 10	1044 ± 70	870 ± 20	1247 ± 50
10^{-8}	1450 ± 20	2465 ± 50	2320 ± 50	2552 ± 70	2610 ± 50	2755 ± 60

[a] Aliquots (100 μL) of the solubilized membranes were incubated with 20 nM [^{3}H]DHA and various concentrations of β-adrenergic agonists and antagonists for 8 min at 30 °C. [^{3}H]DHA binding in the absence of any addition was 29 000 cpm/mL. Results are the mean of three independent experiments made in duplicate.

Table VI: Inhibition of [^{3}H]DHA Binding to the WGA Eluate by Various Concentrations of (−) and (+) β-Adrenergic Ligands[a]

	[^{3}H]DHA binding (cpm/mL)					
ligand concn (M)	(−)-propranolol	(+)-propranolol	(−)-isoproterenol	(+)-isoproterenol	(−)-norepinephrine	(−)-epinephrine
10^{-4}	4 ± 0.02	3010 ± 40	3973 ± 36	7465 ± 20	4832 ± 15	5140 ± 20
10^{-6}	602 ± 20	7224 ± 50	5418 ± 50	7826 ± 30	5757 ± 30	6374 ± 50
10^{-8}	1806 ± 20	12000 ± 35	7826 ± 10	11300 ± 20	8738 ± 18	8944 ± 40

[a] Aliquots (100 μL) of the eluate from the WGA gel were incubated with 20 nM [^{3}H]DHA and various concentrations of β-adrenergic agonists and antagonists for 8 min at 30 °C. [^{3}H]DHA binding in the absence of any addition was 12 000 cpm/mL. Results are the mean of three independent experiments made in duplicate.

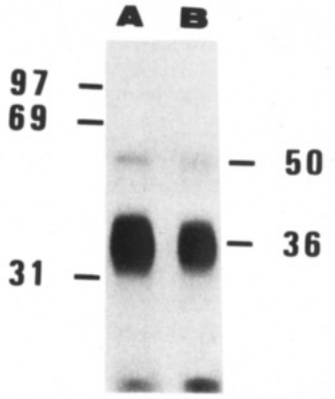


FIGURE 4: SDS–polyacrylamide gel electrophoresis of the purified β-adrenergic receptor from turkey erythrocytes. β-Adrenergic receptor was purified by alprenolol–Sepharose chromatography (lane B) followed by WGA affinity chromatography (lane A). Radioiodination and electrophoresis were carried out as described under Experimental Procedures.

Table VII: [^{3}H]DHA Binding on Solubilized Receptor in the Presence of Several Lectins[a]

lectin	concn (μg/mL)	(−)-[^{3}H]DHA binding (pmol/mg of protein) 4 °C	30 °C
Con A	0	1.05 ± 0.1	1.35 ± 0.11
	25	1.25 ± 0.2	1.26 ± 0.13
	100	1.00 ± 0.08	1.24 ± 0.2
	500	1.2 ± 0.1	1.37 ± 0.18
WGA	0	1.00 ± 0.08	0.49 ± 0.05
	25	0.91 ± 0.08	0.50 ± 0.04
	100	0.93 ± 0.1	0.46 ± 0.03
	500	0.95 ± 0.11	0.49 ± 0.02
PNA	0	0.6 ± 0.05	0.61 ± 0.05
	25	0.47 ± 0.05	0.61 ± 0.04
	100	0.55 ± 0.04	0.60 ± 0.06
	500	0.54 ± 0.03	0.60 ± 0.07
UEA-F	0	1.0 ± 0.02	1.3 ± 0.07
	25	0.9 ± 0.04	1.2 ± 0.07
	100	1.12 ± 0.03	0.95 ± 0.09
	500	1.20 ± 0.06	1.3 ± 0.07

[a] Experimental conditions and [^{3}H]DHA binding were determined as described under Experimental Procedures. Results are the mean of four independent experiments made in duplicate.

cubated with various concentrations of WGA, Con A, UEA-F, and PNA before determination of [^{3}H]DHA binding. As shown in Table VII, these lectins have no effect on [^{3}H]DHA binding.

Discussion

The results presented here show that the β-adrenergic receptor from turkey erythrocyte membranes is a glycoprotein. This is supported by the fact that WGA gel retained 80% of solubilized receptor and 97% of the receptor purified by alprenolol–Sepharose affinity chromatography. These results were obtained by following the activity of [^{3}H]DHA binding of the receptor. They are confirmed by the autoradiography of the SDS electrophoresis of the receptor since Figure 4 shows that the two polypeptides present in the receptor purified by alprenolol–Sepharose chromatography are still present after WGA chromatography. The broadness of the β_1-adrenergic receptor bands can be explained by a microheterogeneity of the carbohydrate chain (Stiles et al., 1984).

The absorption of the β_1-adrenergic receptor on various other lectin columns and its specific elution by free sugars yield information on the structure of the carbohydrate moiety. Thus the retention on the immobilized UEA-F as well as on WGA and specific elution indicate that the carbohydrate moiety contains *N*-acetylglucosamine, fucose, and probably neuraminic acid. Treatment of the membranes with neuraminidase before solubilization and chromatography on LPA and WGA gels revealed the presence of neuraminic acid residues in the glycan. The removal of neuraminic acid unmasked a galactosyl-(β-1,4)-*N*-acetyllactosamine residue, recognized by the immobilized lectin RCA_I and not by PNA. This finding excludes the presence of an unsubstituted galactosyl-*N*-acetylgalactosamine sequence.

The results obtained with Con A–Sepharose demonstrate that at least two α-mannopyranosyl residues are also present since they are essential for the binding of a glycoprotein to Con A. Narasimhan et al. (1978), Ogata et al. (1975), Krusius et al. (1977), and Debray et al. (1981) have studied the interaction of Con A–Sepharose with various oligosaccharidic structures and have shown that this lectin presents a high affinity for a trimannoside core substituted by two *N*-acetyl-β-glucosaminyl residues but that the affinity is reduced when these residues are substituted by galactosyl or sialylgalactosyl residues (Debray et al., 1983). The presence of such an oligosaccharidic structure may explain the elution of the β_1-adrenergic receptor from the column of Con A by a weak concentration of methyl α-mannoside and also by a high concentration of *N*-acetylglucosamine.

Moreover, the elution of the β_1-receptor from Con A gel by a low concentration of mannose indicates that the β-receptor does not bind tightly to this lectin; this suggests a biantennary sialoglycoprotein structure, thus complex carbohydrate chains, and excludes the presence of a bisecting *N*-acetylglucosamine between the two antennae and of an oligomannosidic chain (Narasimhan et al., 1978).

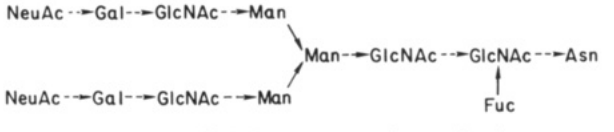
Thus, the carbohydrate moiety of the β_1-adrenergic receptor appears to be different from that described for mammalian β_2-adrenergic receptors (Stiles et al., 1984), which may contain both complex and high-mannose carbohydrate chains. It is interesting to note that both receptors (β_1 and β_2) seem to display microheterogeneity in the carbohydrate chain as evidenced by wide, diffuse bands on SDS–PAGE.

The presence of *N*-acetylglucosamine in the carbohydrate moiety of the β_1-adrenergic receptor indicates an N-glycosidic linkage between *N*-acetylglucosamine and the asparagine residue of the protein; the presence of mannose confirms this type of linkage (Lotan & Nicolson, 1979).

The absence of retention of the β_1-adrenergic receptor on the PNA column after neuraminidase treatment of membranes indicates the absence of *N*-acetylgalactosamine residues and thus suggests the absence of an O-linked carbohydrate chain; albeit the presence of such a linkage cannot be excluded.

It is interesting to note that the insulin receptor has an N-linked carbohydrate composition that coincides with the one proposed for the β_1-adrenergic receptor. It has been suggested that opiate (Gioannini et al., 1982) and muscarinic receptors (Shirakawa et al., 1983) also contain N-linked glycan.

Finally, the structure for the carbohydrate moiety of the β_1-adrenergic receptor from turkey erythrocyte membrane may be proposed:



The exact number of chains was not determined.

The β_1-adrenergic receptor binding site and the structure recognized by the lectins appear to be independent of each other since binding of [^{3}H]DHA on the membrane of turkey erythrocytes is unaffected by relatively high concentrations

of various lectins or by treatment with neuraminidase or Endo H. Similarly, lectins do not interfere with the binding of opiates (Gioannini et al., 1982) or muscarinic ligands to their respective receptors (Shirakawa et al., 1983). In contrast, some lectins modify the binding of insulin (Caron et al., 1983; Cuatrecasas, 1973b; Sorge & Hilf, 1981), EGF (Carpenter, 1977), and NGF (Buxser et al., 1983; Vale & Shooter, 1982) to their respective receptors.

No data are available concerning the role of carbohydrates in the binding activity of the mammalian β_2-adrenergic receptor, recently characterized as a glycoprotein (Stiles et al., 1984).

The present study reveals the potential usefulness of lectin chromatography for a partial purification of the β-adrenergic receptor: the high retention on WGA gel constitutes a simple one-step method for a preliminary purification since the specific [^{3}H]DHA binding activity was increased 22-fold in the glycoprotein fraction as compared to the initial membrane protein preparation.

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Registry No. DHA, 59624-90-7; (−)-propranolol, 4199-09-1; (+)-propranolol, 5051-22-9; (−)-isoproterenol, 51-31-0; (+)-isoproterenol, 2964-04-7; (−)-norepinephrine, 51-41-2; (−)-epinephrine, 51-43-4.

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