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Lectin Binding to the Porcine and Human Ileal Receptor of Intrinsic Factor-Cobalamin

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The purified porcine receptor for the intrinsic factor-cobalamin complex bound to concanavalin A, lentil lectin and wheat germ lectin covalently coupled to Sepharose and was eluted with the corresponding soluble sugars. In contrast, human intrinsic factor bound efficiently to concanavalin A, to some extent to lentil lectin, but only slightly to wheat germ agglutinin. The binding of IF-Cbl to the receptor was inhibited when the receptor was pre-incubated with soluble wheat germ agglutinin, with an inhibition constant estimated to be 1.9 μmol/l. After transfer of the purified receptor from SDS-PAGE to Immobilon, ligand blotting of the purified receptor with iodinated lectin showed that concanavalin A and lentil lectin bound to three (75, 56 and 43 kDa) components but that wheat germ agglutinin bound only to the 75 kDa component. These results showed that the α subunit of the receptor could bind to wheat germ agglutinin, resulting in an inhibition of its binding with intrinsic factor. Both binding sites of intrinsic factor and of wheat germ agglutinin could be located near to each other.

The receptor for intrinsic factor-cobalamin (IF-Cbl) is located in the brush border membranes of the distal small intestine (ileum) and binds the IF-Cbl complex in the presence of calcium ions at neutral pH. The binding is followed by translocation of Cbl into the enterocyte by a mechanism which is not fully understood. The receptor for IF-Cbl has been isolated from pig [1], man [1], dog [2], guinea pig [3] and rat [4].

Numerous membrane-bound proteins, including several receptors, are glycoproteins [5-8]. The porcine receptor for IF-Cbl consists of two subunits, which have been stained with Schiff's reagent and thus seem to contain carbohydrates [1]. The carbohydrates can be lost in part during the purification procedure. This could explain that the canine receptor purified by Seetharam *et al.* [2] contained less than 1% of amino-sugars and did not stain for carbohydrate.

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Human IF contains about 14% carbohydrates [9]. The carbohydrates of both the receptor and IF could be involved in the calcium-dependent binding of the molecules [10]. To determine which part of the receptor is glycosylated is, therefore, of importance in understanding its function.

In the present work, we have studied the binding of IF receptor from hog and human intestine to soluble and Sepharose-bound lectins. Three lectins were tested: concanavalin A, lentil lectin and wheat germ agglutinin. Our purpose was not only to compare the binding of three different lectins to the receptor but also to determine if the lectins modify the binding of intrinsic factor to its receptor.

Experimental Procedures

Materials

Cyano[^{57}Co]cobalamin (389 kBq/ml, 0.0477 µg/ml) was purchased from Amersham, UK; concanavalin A (ConA), lentil lectin and wheat germ lectin (WGA) as well as ConA-Sepharose 4B, lentil lectin-Sepharose 4B and WGA-Sepharose 6B from Pharmacia Uppsala, Sweden; *N*-acetylglucosamine from Sigma, St Louis, MO, USA, and α-methylmannoside from Fluka AG, Buchs, Switzerland. Human gastric juice was collected after pentagastrin stimulation, centrifuged to remove particles and stored at -20°C in aliquots [11]. Its Cbl-binding capacity was measured by the hemoglobin-coated charcoal method [12].

Preparation of Receptor Extract

Fresh porcine ilea were washed with 0.1 M sodium acetate buffer, pH 5.0, containing 0.15 M NaCl, 0.4 mM phenylmethylsulfonylfluoride (PMSF) and 1.5 mM NaN_3, to remove endogenous IF bound to the receptor. The mucosa was scraped off with a glass slide and homogenized in the same buffer. After centrifugation (10,500 x *g*, 30 min) the pellet was washed three times with 0.1 M Na-K-phosphate buffer, pH 7.4, containing 0.15 M NaCl, 0.4 mM PMSF and 1.5 mM NaN_3 and finally homogenized in 5 mM Tris-HCl buffer, pH 8.0, containing 0.2% Triton X-100 (by vol), 0.4 mM PMSF and 1.5 mM NaN_3 (extracting buffer). One liter of extracting buffer was used for 150-200 g of scraped mucosa. After sonication the mixture was incubated at +4°C on a rotating mixer for three days. Then $CaCl_2$ was added to 2.5 mM concentration and the extract was centrifuged (50,000 x *g*, 2 h) to remove insoluble cell debris.

One human ileum was obtained from a brain-dead person with beating heart used as a kidney-donor (permission obtained from the Ethics Committee of the Helsinki University Central Hospital). Human receptor extract was prepared as described above.

Purification of the Porcine Receptor

This was performed as previously described [1, 14] using Cbl-Sepharose gel saturated with human intrinsic factor as affinity chromatography medium. The physico-chemical properties of the purified receptor are described in refs. 1 and 14.

Receptor Activity Assay

Receptor activity was determined by incubating the sample with human gastric juice saturated with [^{57}Co]Cbl following the blocking of haptocorrin with cobinamide, and filtering the mixture through a gel filtration column in 20 mM Tris-HCl buffer, pH 7.4 containing 0.05 % Triton X-100 (by vol), 0.15 NaCl, 1 mM $CaCl_2$ and 1.5 mM NaN_3 (receptor buffer). One of the following columns were used : a conventional Sephadex G-200 column (1.5 x 90 cm) at a flow rate of 10 ml/h, or a HPLC system (Gilson, France) with a Superose 6 or 12 column (HR 10/30, Pharmacia) at a flow rate of 0.5 ml/min. Totally excluded radioactivity reflects receptor activity, i.e.[^{57}Co]Cbl bound to the receptor *via* IF [14]. At low pH or in the presence of 10 mM EDTA, no radioactivity emerged in the void volume. Receptor activity was expressed as pmol Cbl bound to receptor *via* IF.

Binding of Receptor to Lectin Sepharoses

ConA-, lentil lectin- and WGA-Sepharoses were washed with receptor buffer before use. A 1 ml sample of affinity-purified porcine receptor (0.2 mg/ml in receptor buffer) was incubated overnight at +4°C with 250 µl of each gel. The gels were then washed with receptor buffer to remove unbound receptor. ConA- and lentil lectin-Sepharoses were eluted by incubating them with 0.5 M α-methylmannoside, and WGA-Sepharose with 0.5 M *N*-acetylglucosamine in receptor buffer. Receptor activity was measured in each step (before incubation with gel, in the supernatant after incubation and in the eluate). Samples of receptor extract (5 ml) were treated in the same way with one ml of each gel. The binding of IF-receptor to ConA was also studied by column affinity chromatography. Hog ileal receptor extract (30 ml) was filtered through a ConA-Sepharose column (1.6 x 25 cm) at a flow rate of 0.5 ml/min. The column was successively washed with 120 ml of 0.02 M α-methylmannoside, 0.2 M α-methylmannoside and 1.0 M α-methylmannoside in receptor buffer containing 1 mM $CaCl_2$ and 1 mM $MgCl_2$ and with 120 ml of 0.05 M sodium acetate buffer pH 4.5 containing 10 mM EDTA. The fractions eluted at each step were concentrated to 2 ml and used for receptor activity determination as described above.

Binding of IF to Lectin Sepharoses

Each gel (100 µl) was washed and incubated with human gastric juice containing 0.64 pmol IF (haptocorrin was blocked with cobinamide) saturated with cyano[^{57}Co]Cbl. The gels were washed with receptor buffer until no radioactivity was detected and eluted as described in the previous section. As a control [^{57}Co]Cbl was incubated alone with each gel.

Binding of IF and of Receptor Extract to Soluble Lectins

Ten microliters of human gastric juice (Cbl-binding capacity corresponding to IF 37 pmol/l), 10 µl cobinamide (10 µg/ml) and 10 µl [^{57}Co]Cbl were incubated in triplicate at room temperature for 30 min. ConA, lentil lectin or WGA (respectively 1.2, 2.3 and 3.6 nmol/ml in receptor buffer, total volume of 1 ml) were added in each sample and incubation was continued overnight. Each mixture was separately applied to a Superose 6 FPLC column. The same experiment was repeated by incubating the lectins with 0.1 ml of receptor extract overnight, before adding them to human gastric juice. The samples were then filtered in gel filtration for receptor activity determination.

Table 1. Binding of the ileal receptor of intrinsic factor to concanavalin A, lentil lectin and wheat germ agglutinin coupled to Sepharose.

Incubation medium	Purified hog receptor[a] (pmol)			Human receptor extract[a] (fmol)		
	Total	Adsorbed[b]	Eluted[c]	Total	Adsorbed[b]	Eluted[c]
Concanavalin A	3.75	3.75 (100)	1.82 (48.5)	66	41 (62.1)	2 (4.9)
Lentil lectin + 0.5 M α-methyl-mannoside	3.75	0.00 (0)	3.75 (100)	-	-	-
Lentil lectin	3.75	3.38 (90.1)	1.96 (58.0)	66	52 (78.8)	49 (94.2)
Wheat germ agglutinin	5.20	5.05 (97.1)	1.24 (24.5)	66	30 (45.4)	27 (90.0)
Wheat germ agglutinin + 0.5 M *N*-acetyl-glucosamine	5.20	0.00 (0)	5.20 (100)	-	-	-

[a] Assay of the receptor was performed in duplicate on Superose 6 gel filtration and corresponded to the amount of intrinsic factor-cyano[^{57}Co]cobalamin which was bound to the receptor as described in the Methods section.
[b] Adsorbed receptor was estimated from the difference between total receptor and receptor remaining in the soluble phase. Values in parentheses correspond to the percentage of total receptor which was adsorbed to the lectin gel.
[c] Elution of the receptor was studied in the presence of 0.5 M α-methylmannoside for concanavalin A and lentil lectin and in the presence of 0.5 M *N*-acetylglucosamine for wheat germ agglutinin. Values in parentheses are the percentage of adsorbed receptor that was eluted.

Binding of Receptor to Soluble WGA and Inhibition of IF-Receptor Complex Formation by WGA

The receptor activity of the porcine extract was studied in the presence of increasing amounts of WGA. In the first series 0.5 ml of receptor extract was incubated with 0-24 nmol WGA at room temperature overnight. Human gastric IF (0.37 pmol) saturated with [^{57}Co]Cbl (as described above) and receptor buffer were added, in a total volume of 1 ml. After 60 min incubation at room temperature, the mixture was filtered through a Superose 12 FPLC column. Another series was carried out using 0.1 ml receptor extract and 0-12 nmol WGA. The column was washed after each run with 0.05 M sodium acetate buffer, pH 4.0, containing 0.25 M *N*-acetylglucosamine and 2 mM EDTA to remove any soluble lectin which could be retained into the column.

The results of the FPLC runs were confirmed using density gradient ultracentrifugation as described previously [15, 16]. The incubation mixtures were prepared as described above using 0.2 ml receptor extract and 0-28 nmol WGA and were applied on a continuous 10-

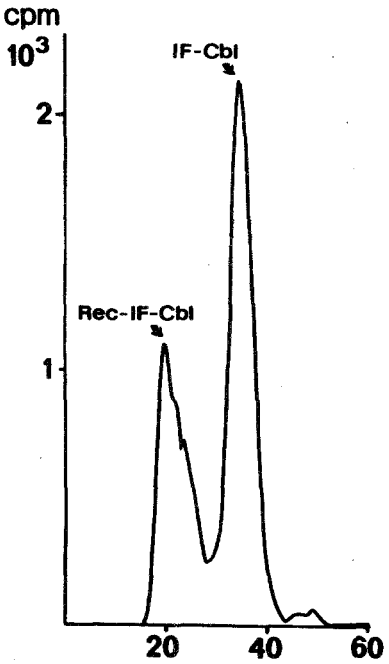

Figure 1. Elution profile on Superose 6 gel filtration of receptor extract (500 µl) incubated with 0.4 pmol of intrinsic factor-CN[^{57}Co]cobalamin. The receptor-intrinsic factor-cobalamin (rec-IF-Cbl) complex and the free saturated intrinsic factor were eluted with a retention time of 19 min and 36 min respectively. The receptor concentration corresponded to the amount in pmol of intrinsic factor which was eluted in the 19 min peak. No modification of the elution profile was observed when the incubation and the filtration were performed in the presence of either 0.5 M *N*-acetylglucosamine or 0.5 M α-methylmannoside.

30% sucrose gradient in receptor buffer (12 ml per tube). A Beckman-Spinco preparative ultracentrifuge was used with a SW 41 rotor. The runs (30,000 rev./min, 25 h) were performed at + 4°C. Fractions (0.2 ml) were collected and analyzed for radioactivity.

Ligand Blotting of Purified Receptor with Radiolabelled Lectins

ConA, lentil lectin and WGA were iodinated using the method of Markwell [17] as described recently for IF [9]. Samples of purified receptor (about 20 µg) were run in 7.5% PAGE, according to the method of Laemmli [18]. After electrophoresis, the proteins were transferred to an Immobilon membrane (Millipore, USA) using a Bio-Rad electroblotting system, during 17.5 h (30 V, maximum intensity 300 mA). After transfer, the gel was stained with silver; no bands were detected, indicating that the transfer was efficient. The Immobilon membrane was then cut into strips, wetted quickly with pure methanol and washed with distilled water for 5 min. Unspecific binding sites were blocked by incubating the strips in 20 mM Tris-HCl buffer pH 7.4 containing 0.15 M NaCl and 1% (w/v) hemoglobin for 60 min at 37°C. The strips were then washed with the same buffer containing 0.1 % (w/v)

Table 2. Binding of human gastric intrinsic factor-CN[^{57}Co]Cbl complex to concanavalin A, lentil lectin and wheat germ agglutinin coupled to Sepharose[a].

Lectin-Sepharose	Adsorbed[b] (pmol)	Eluted[c] (pmol)
Concanavalin A	0.62±0.02 (96.9)	0.60±0.01 (96.8)
Lentil lectin	0.24±0.07 (37.5)	0.23±0.07 (95.8)
Wheat germ agglutinin	0.08±0.05 (12.5)	n.d.[d]

[a] Data are expressed as mean ± SD (n=4).
[b] Adsorbed intrinsic factor-Cbl complex was estimated from the difference between total amount (0.64 pmol) and the amount remaining in the soluble phase. Values in parentheses are the percentage of total amount adsorbed to the lectin gel.
[c] Elution was performed with 0.5 M soluble sugar as described in Table 1. Values in parentheses are the percentage of adsorbed intrinsic factor-Cbl that was eluted.
[d] n.d. = not determined.

hemoglobin for 3 x 5 min and incubated with the iodinated lectins in 20 ml of buffer containing 0.1 % (w/v) hemoglobin for 60 min at room temperature. After washing them again successively with Tris-HCl buffer containing 0.1% hemoglobin (2 x 5 min) and with Tris-HCl buffer without hemoglobin (2 x 5 min), the strips were dried and subjected to autoradiography for three days.

Results

Studies with Lectin-Sepharoses

As shown in Table 1 the adsorption of the purified receptor to the lectin-gels was nearly complete (97-100%). Elution of the gels with the corresponding sugars, i.e. ConA- and lentil lectin-Sepharoses with α-methylmannoside, and WGA-Sepharose with *N*-acetylglucosamine, resulted in 24-58% elution of the bound receptor activity. Control experiments showed that 0.5 M α-methylmannoside or *N*-acetylglucosamine had no effect on receptor activity (Fig. 1). As shown in Table 1, the adsorption of 45-79% of the total receptor activity from the human receptor extract to the lectin gels was also obtained. Elution of the bound receptor from lentil lectin- and WGA-Sepharose was nearly complete (94 and 90% of the bound activity, respectively). On the contrary, only 5% of the bound receptor was eluted from ConA-Sepharose with 0.5 M α-methylmannoside. In control experiments free [^{57}Co]Cbl was incubated with lectin gels. No radioactivity was bound by any of the gels. No binding of the IF-receptor to lectin-Sepharoses was observed in the presence of *N*-acetylglucosamine or α-methylmannoside, under identical experimental conditions (Table 1).

The binding of human gastric IF to the lectin Sepharoses differed from that of the receptor. As shown in Table 2, IF bound completely to ConA-Sepharose but only partially to lentil lectin-Sepharose. The interaction with WGA-Sepharose was weak; not more than 13% was

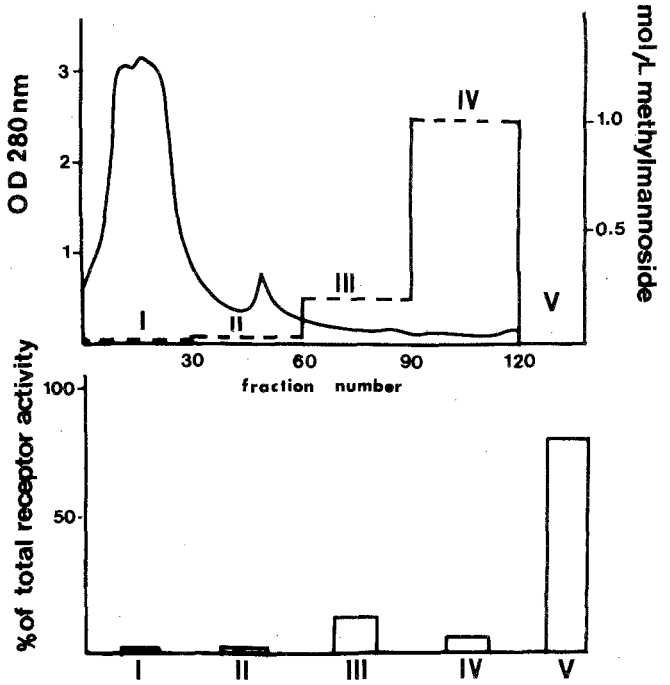

Figure 2. Elution profile of porcine ileal mucosal extract on ConA affinity chromatography. The column (1.6 x 25 cm) was eluted successively with 0.02 M α-methylmannoside (step I), 0.2 M α-methylmannoside (step II), 1.0 M α-methylmannoside (step III) and washed with 0.05 M sodium acetate pH 4.5, containing 10 mM EDTA. The fractions (4 ml) collected at each step were pooled, ultrafiltered to 2 ml and assayed for receptor activity. The receptor activity (12.8% and 6.0%) was eluted in steps III and IV, respectively. The remaining activity was removed by washing the column at low pH, in step V.

bound. Elution of the bound IF from ConA- and lentil lectin-Sepharoses with 0.5 M α-methylmannoside was total. Elution of IF from WGA-Sepharose was not studied because so little radioactivity was bound by the gel.

A concanavalin A affinity column was loaded with receptor extract and eluted stepwise with different concentrations of α-methylmannoside. The elution profile is shown in Fig. 2. About 12.8 and 6.0% of the receptor activity was eluted when the column was treated with 0.2 M and 1 M α-methylmannoside, respectively (Fig. 2). The remaining activity was removed by washing the column with 0.05 M sodium acetate buffer pH 4.5 containing 10 mM EDTA.

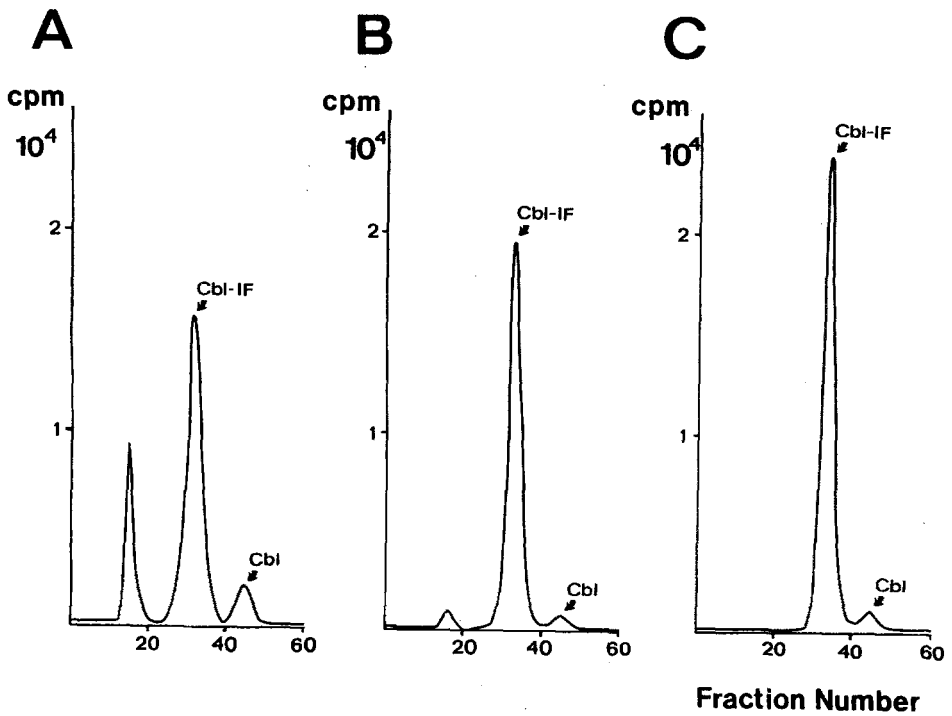

Figure 3. A. Elution profile on Superose 12 gel filtration of gastric intrinsic factor (0.4 pmol) saturated with CN[^{57}Co]cobalamin and incubated with 1.2 nmol/ml of concanavalin A. About 24% of intrinsic factor was eluted in the void volume (fraction 17), corresponding to its binding with the lectin.
B. The same experiment performed with 2.3 nmol/ml of lentil lectin instead of concanavalin A, showing that about 3% of intrinsic factor was eluted in the void volume, as bound to the lectin.
C. The same experiment performed with 3.6 nmol/ml of wheat germ agglutinin, showing that no complex of lectin-intrinsic factor was eluted in the void volume.

Studies with Soluble Lectins

Binding of human IF to soluble lectins was studied by FPLC. Human gastric juice saturated with [^{57}Co]Cbl and cobinamide gave only two radioactive peaks when run on a Superose column, one corresponding to IF-Cbl and the other to free cobalamin. When this mixture containing 0.4 pmol of saturated IF was incubated either with 1.2 nmol/ml of ConA or 2.3 nmol/ml of lentil lectin before the run, a peak was observed in the void volume position, indicating complex formation between the lectins and IF, as shown in Fig. 3. No peak was observed when the experiment was performed with 3.6 nmol/ml of WGA (Fig. 3). When soluble concanavalin A or lentil lectin was incubated with 100 µl receptor extract prior to incubation with saturated labeled IF, no effect was observed on the elution position and size of the void volume peak. In contrast, a 54% decrease of the peak of receptor IF-Cbl was observed on gel filtration when the receptor extract was treated with WGA.

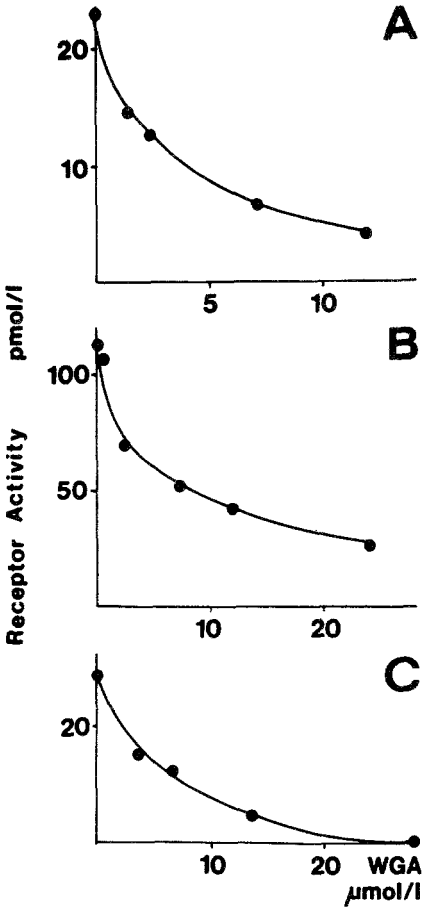

Figure 4. A. Inhibition of intrinsic factor receptor activity of intestinal mucosal extract (100 μl) in the presence of increasing concentrations (0-12 nmol/ml) of wheat germ agglutinin (WGA). The assay was performed by Superose 6 gel filtration (see the Methods section). No inhibition was observed when the experiment was performed in the presence of 0.5 M *N*-acetylglucosamine.
B. The same experiment with 500 μl of intestinal mucosal extract in the presence of 0-24 nmol/ml of WGA. The receptor assay was also performed by Superose 6 gel filtration.
C. The same experiment with 200 μl of intestinal mucosal extract in the presence of 0-28 nmol/ml of WGA. The receptor assay was performed by sucrose-density ultracentrifugation as described in the Methods section.

Four FPLC control runs were made to verify that the binding of the receptor to WGA was caused by carbohydrates and not by unspecific forces. Receptor extract was incubated alone, with WGA, with *N*-acetylglucosamine and with both. *N*-Acetylglucosamine had no effect on receptor activity. In the presence of both *N*-acetylglucosamine and WGA the receptor activity was still unchanged, but when only WGA was added a clear decrease of

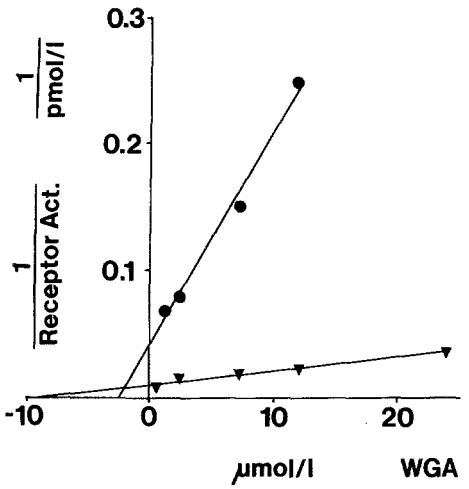

Figure 5. Dixon plot of the inhibition of wheat germ agglutinin on the intrinsic factor-receptor activity of porcine mucosal extract. Two series were performed with 100 (●) and 500 (▼) µl of receptor extract, and receptor activity was assayed as shown in Figs. 1 and 4. The inhibition constant (Ki) was estimated to be 1.9 µmol/l.

receptor activity was observed. When the extract was first incubated with [^{57}Co]Cbl-IF followed by WGA, it was found that even the preformed Cbl-IF-receptor complex was split by WGA.

When porcine receptor extract (100 µl or 500 µl) was incubated with increasing concentrations of WGA (0-36 nmol/ml) prior to incubation with[^{57}Co]Cbl-IF, the decrease of the receptor activity determined on gel filtration corresponded to typical curves of competitive inhibition (Fig. 4). A Dixon plot (1/Receptor activity vs. the amount of WGA) was drawn from the results of the two gel filtration series with either 100 µl or 500 µl of receptor extract and WGA (Fig. 5), corresponding to an inhibition constant (Ki) estimated to be 1.9 µmol/l.

The result was confirmed using sucrose gradient (10-30% w/v) ultracentrifugation (Figs. 5 and 6). Again, the greater the amount of WGA added, the lower was the peak corresponding to the receptor-IF-Cbl complex. Fig. 6 presents typical profiles of the run.

The SDS-PAGE of the purified porcine receptor showed four main bands with M_r of 75, 60, 56 and 43 kDa. Such an electrophoresis pattern has been previously observed for this receptor [14]. After transfer of the protein material to an Immobilon membrane, the bands with the M_r of 75, 56 and 43 kDa were easily identified by blotting with iodinated ConA and lentil lectin (Fig. 7). In contrast, only the band with M_r 75 kDa was identified with WGA (Fig. 7). No band was detected when the SDS-PAGE gel was stained with silver after transfer, indicating that the transfer was nearly complete.

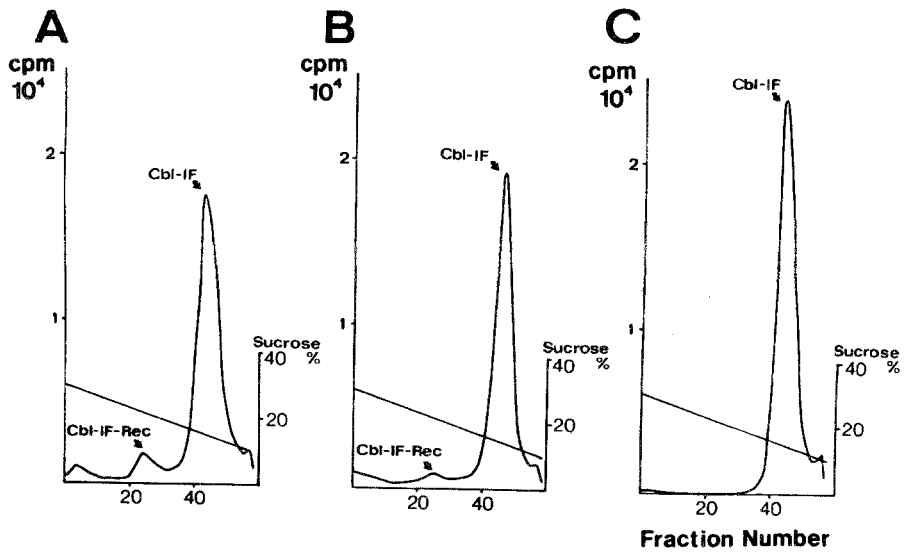

Figure 6. A. Sucrose gradient ultracentrifugation of porcine intestinal mucosal extract (200 µl) incubated with human gastric intrinsic factor-cyano[^{57}Co]cobalamin (0.4 pmol). The sample volume was 1 ml. The run was performed as described in the Methods section, for 25 h at 4°C. Two peaks were collected, the first one corresponding to intrinsic factor-cobalamin-receptor complex (Cbl-IF-rec) and the second to intrinsic factor-cobalamin (IF-Cbl).

B. The same experiment, except that the porcine mucosal extract was incubated overnight with 5.5 nmol of wheat germ agglutinin (WGA) prior to incubation with human gastric intrinsic factor-cyano[^{57}Co]cobalamin. A 57% decrease of the receptor-intrinsic factor peak was observed.

C. Control experiment in which the sample was 0.4 pmol gastric intrinsic factor-cyano[^{57}Co]cobalamin incubated overnight with 6.5 nmol WGA in a total volume of 1 ml. No binding of WGA to intrinsic factor was observed.

Discussion

The present study shows that both the porcine and the human receptors for Cbl-IF bind to ConA-, lentil lectin- and WGA-Sepharoses. Because of the very low concentration of the human receptor, only crude extract was used in the studies on this receptor. This may explain why the three lectin gels bound only 45-79% of the total receptor activity from this extract; other glycoproteins probably competed for the binding sites of the gels. Incubation of lentil lectin- and WGA-Sepharoses with the corresponding sugars resulted in complete elution of the bound human receptor activity. This indicates that the binding was sugar-specific showing that these two lectin-solid phases could be used for partial purification of the receptor. In contrast, the binding to ConA-Sepharose was very strong, as α-methylmannoside only eluted traces of receptor activity. No definite explanation can be provided from these data. One could hypothesize that the binding was not specific or that the affinity of the receptor for ConA was very strong.

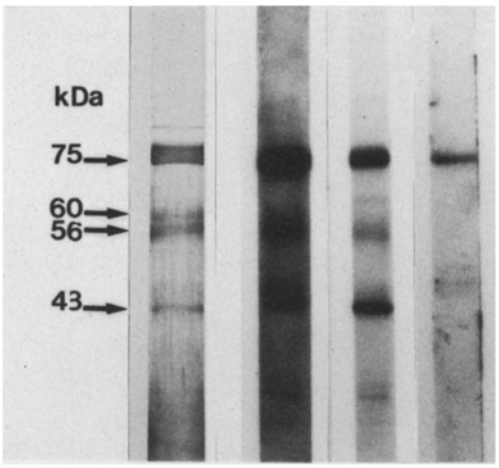

Figure 7. Lectin blotting of SDS-PAGE of 20 μg of purified porcine receptor after transfer to an Immobilon membrane. Lane A: the Immobilon membrane was stained with colloidal gold. Four main bands were identified with M_r of 75, 60, 56 and 43 kDa. Lanes B, C and D show the blotting of the Immobilon membrane with iodinated concanavalin A, lentil lectin and wheat germ agglutinin (WGA). The strips were exposed to X-ray film for three days. Lanes B and C showed that the 75, 56 and 43 kDa bands were blotted with both concanavalin A and lentil lectin. In contrast, only the 75 kDa band was blotted with WGA, in lane C (A, B, C, D from right to left).

As IF is known to be a highly glycosylated protein [9], the question arises whether the binding of the receptor to the lectin Sepharoses was mediated by contaminating IF complexed with the receptor, in the soluble extract. However, if IF were the only part of the receptor-IF complex participating in the lectin binding, the preparation should have contained only holo-receptor (receptor saturated with IF-Cbl) to make the observed binding possible. This is not likely, since: 1) the receptor assay in gel filtration only measured the unsaturated receptor; and 2) the intestines were washed with low pH buffer to remove endogenous IF before solubilization and purification of the receptor. The purified porcine receptor bound nearly totally to the gels but we did not succeed in eluting completely the receptor activity with specific sugars. Removal of the receptor activity by ConA affinity chromatography was mostly attained by elution with low pH and with cation chelator (Fig. 2). The affinity of glycans for ConA has been studied by Debray *et al*, for several examples of *N*-acetyl-lactosamine- or oligomannoside-type oligosaccharides [19, 20]. In the present work, no conclusion can be provided concerning the carbohydrate structure of the receptor. For example, Kornfeld and Ferris showed that the most active part of an *N*-linked glycan in the binding to ConA is not an α-mannose residue in a terminal position but the disaccharide GlcNAcβ1-2Manα1-3 or -6 [21].

IF and the receptor behaved differently in their binding to lectin-Sepharose. Both the porcine and human receptor bound well to the three lectins. On the other hand IF bound to ConA-Sepharose better than to lentil lectin-Sepharose and it bound only poorly to WGA-Sepharose. On gel filtration, the binding of IF-Cbl to both the receptor (Fig. 1) and the soluble ConA and lentil lectin resulted in a shift of a part of the labelled IF-Cbl to the void volume, corresponding to the formation of aggregates. It was not therefore possible to determine if the void volume peak corresponded to a receptor-IF complex or to a lectin-IF aggregate when the receptor was incubated consecutively with ConA (or lentil lectin) and with IF-Cbl.

The results obtained with WGA were easier to interpret since: 1) soluble WGA did not form any aggregate with IF-Cbl on gel filtration; and 2) the incubation of increasing concentrations of WGA with the receptor decreased the formation of IF-Cbl aggregates when incubating the mixture with labeled IF-Cbl. The inhibition of the binding of IF to its receptor in the presence of WGA was therefore provoked by the binding of WGA to the receptor and not to IF. It also means that the binding sites for IF and WGA, even if not the same, are sterically situated close enough to each other to prevent the binding of IF, when WGA is present in excess. The 75 kDa component of the purified receptor observed in SDS-PAGE corresponds to the α subunit, as described previously [1, 14]. It has been shown that the α subunit of the hog receptor is the part of the receptor which binds to IF and that it is located outside the plasma membrane [1, 14]. It is therefore highly probable that WGA binds to this α subunit since: 1) it can inhibit the complexing of IF with the receptor; and 2) it binds to a component having the same M_r as the α subunit (75 kDa) in lectin blotting of the SDS-PAGE of purified receptor. The other components obtained on SDS-PAGE (43 and 56 kDa) were blotted by ConA and lentil lectin, but not by WGA (Fig. 7). This could mean that both α and β subunits are glycosylated but that differences exist in the structures of the glycans.

The dissociation *in vitro* of the IF-Cbl-receptor complex in an excess of WGA led to the question of the effect of WGA *in vivo*. One should investigate, from a nutritional point of view, if WGA agglutinin feeding is able to induce a malabsorption of Cbl in mammals and also if WGA can be internalized into the enterocytes *via* the IF receptor, as previously observed for IF [22].

In conclusion, lectins bind to the ileal receptor of IF and can be used for partial purification of the receptor from soluble extracts. In addition, it was observed that WGA can inhibit the complexing of IF to its receptor. From lectin blotting after SDS-PAGE of the purified receptor, it was concluded that both α and β subunits of the receptor were glycosylated, but that the glycans were different.

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