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Properties of odour-binding glycoproteins from rat olfactory epithelium

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The specific membrane glycoproteins with high affinity for camphor and decanal were isolated from rat olfactory epithelium. Antibodies to these glycoproteins inhibited both the electroolfactogram and the binding of odorants. The enzyme immunoassay has shown these glycoproteins to be present in the olfactory epithelium of rat, mouse, guinea-pig and hamster but not in that of frog and carp. The molecular mass of the odour-binding glycoproteins from rat olfactory epithelium solubilized by Triton X-100 was approx. 140 kDa. They consisted of two subunits (88 and 55 kDa). The 88 kDa subunit was capable of binding odorants. The data obtained suggest that the glycoproteins isolated have some properties that make them plausible candidates for olfactory receptor molecules.

Introduction

The odorant binding to receptor molecules of olfactory sensory neurons is widely believed to be the first step in vertebrate chemoreception. However, the molecular identity of such molecules has not been clearly established. Two methods of selection of 'candidates' for possible olfactory receptor molecules are mainly used. The first one occurs through the specific binding of radiolabeled odorants, as in reception of hormones and neurotransmitters [1–5]. The second one is to reveal the proteins specific to olfactory cilia which are believed to contain the molecular sensory apparatus [6–9]. Using these methods separately, a number of candidates for receptor molecules has been suggested, but satisfactory identification has not been achieved. It is clear that only the simultaneous use of the main criteria (described below) would allow the identification of the receptor molecules [10].

In this study the description of isolating the proteins with receptor properties from olfactory epithelium is given. The following criteria were used to select the olfactory receptor candidates: (i) specific binding of odorants, (ii) tissue specificity, (iii) specific recognition by function-modulating agents (antibodies).

The ability of receptors to bind ligands was used as an initial step to reveal receptor candidates. Therefore, the purification of the receptor candidates was performed without denaturating agents (SDS, urea), because these agents have been shown to inhibit the odorant binding [11].

In this study polyclonal antibodies were used as function modulating agents of odour-binding proteins from rat olfactory epithelium. Monoclonal antibodies interact with the definite protein determinants, and an intensive screening programme is often necessary to obtain antibodies for the functionally important determinant. In contrast, polyclonal antibodies contain a set of immuno-

Abbreviations: FITS, fluorescein isothiocyanate; SDS, sodium dodecyl sulphate.

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globulins for different determinants including the functionally important ones. This essentially simplifies the study of the odour-binding proteins isolated.

A rather high amount of antigen was required to produce rabbit antibody against receptor candidate. Thus, rats were used as macrosmatics. The isolated cilia preparation could considerably simplify the purification of receptor candidate [12,13], but the preparative isolation of rat olfactory cilia was evidently rather complicated. Therefore, a crude preparation (whole tissue) was used as a starting material.

Materials and Methods

Materials. [^{3}H]Camphor (10 Ci/mmol) and [^{3}H]decanal (25 Ci/mmol) were synthesized in Institute of Molecular Genetics, U.S.S.R. Academy of Sciences. [^{3}H]Camphor, [^{3}H]decanal and unlabeled odorants were purified by preparative gas-liquid chromatography. Chemicals for enzyme immunoassay (EIA) are from Amersham (U.K.). Chemicals for gel electrophoresis are from Reanal (Hungary). Ultrogel AcA-34 is from LKB (Bromma, Sweden).

Isolation of odour-binding glycoproteins. 800 Wistar rats of both sexes (180–250 g) were used. $20\,000 \times g$ pellets of rat olfactory epithelium and other tissues were prepared as previously described [11]. Membrane proteins were solubilized by 0.3% Triton X-100 in 50 mM Tris-HCl buffer (pH 7.2) and detergent-insoluble proteins were separated by centrifugation ($20\,000 \times g$ for 1 h, twice). One-quarter of the solubilized proteins was applied to Ultrogel AcA-34 column. The column (3×150 cm) was equilibrated and eluted with 50 mM Tris-HCl buffer (pH 7.2) containing 0.1% Triton X-100 and 0.1 M NaCl. The fractions M_r 120 000–150 000 from four chromatographic runs were pooled and dialyzed against 10 mM Tris-HCl buffer (pH 7.2) containing 0.1% Triton X-100. After dialysis, proteins were isoelectrofocused (IEF) in Sephadex G-75, superfine (2% ampholine, pH range 3–10). The fractions of p*I* 4.7–5.1 were collected and isoelectrofocused again in Sephadex G-75 (2% ampholine, pH range 4–6). The fraction of p*I* 4.9 (200 μg of protein) was used to produce the rabbit antibody. Prior to the first antigen injection the animal was bled from ear veins, serum was separated from the blood and the IgG fraction was obtained using Protein A-Sepharose (control IgG). For immunization, 100 μg of protein in 0.5 ml of saline were emulsified with 0.5 ml of Freund complete adjuvant immediately prior to injection. The emulsion was injected into each hind foot pad of the rabbit. After 5 weeks the animal was given a booster injection identical to the first immunization. After 10 days the animal was bled from ear vein. The serum titer was 1/6000, tested by ELISA using anti-rabbit IgG peroxidase conjugate developed in goat. IgG fraction was obtained using a Protein A-Sepharose affinity column. The IgG obtained was incubated with the suspension of $20\,000 \times g$ pellet of lung (10 mg IgG with 25 mg protein of $20\,000 \times g$ pellet) in 0.1 Tris-HCl buffer (pH 7.2) for 3 h at 4° C. The suspension was centrifuged at $10\,000 \times g$ for 30 min and the IgG obtained (IgG*) was purified again using Protein A-Sepharose affinity column. IgG* was coupled to CNBr-activated agarose by the standard method and IgG*-agarose affinity column was used for purification of the candidates for olfactory receptor molecules. The M_r 120 000–150 fraction of the Triton X-100 extract of $20\,000 \times g$ pellet was applied to the IgG-agarose column, which was equilibrated with 50 mM Tris-HCl buffer contained 0.1% Triton X-100 and 0.5 M NaCl. The bound proteins were eluted with 20 mM glycine-HCl buffer (pH 2.8) containing 0.5 M NaCl and 0.1% Triton X-100. According to SDS-gel electrophoresis, the fractions from both types of sample applied to the affinity column were qualitatively identical. 50–80 μg of odour-binding glycoproteins (gp88/55, see Results) was obtained by IgG*-agarose column from Triton X-100 extract of $20\,000 \times g$ pellet from 100 animals.

Electrophoresis. Electrophoresis in 10–20% polyacrylamide gels in the presence of SDS and 2-mercaptoethanol was carried out using a discontinuous buffer system, according to the method of Laemmli [17]. Polypeptides were visualized by the silver staining method [18]. Glycoproteins were identified directly on the gel by the lectin overlay method [19] with fluoresceinated concanavalin A.

Analytical isoelectric focusing. The concentrations of acrylamide, ampholytes and Triton X-100

were 5.0, 1.0 and 0.1%, respectively. The gel was polymerized by ammonium persulfate. The sample (300–600 μg of protein) was added directly to acrylamide solution prior to gel polymerization. The concentration of Triton X-100 along the gel after focusing was determined at 280 nm.

Binding assay. The gel chromatography: the sample (volume, 1 ml; protein, 1 mg/ml) was incubated with [^{3}H]camphor or [^{3}H]decanal for 1 h at 4°C before it was applied to the column. The final concentration for both labeled ligands was $5 \cdot 10^{-10}$ M. The radioactivity of the fractions was determined with a scintillation counter.

For isoelectrofocusing, the binding assay was performed as described earlier [2]. Gel slices were incubated in 10 ml 0.1 M Tris-HCl (pH 7.4) containing $5 \cdot 10^{-10}$ M [^{3}H]camphor for 5 h at 4°C. Gel slices were washed twice in water, transferred to counting vials and 1 ml of methanol was added, followed (10 h later) by 10 ml Bray's solution.

To study the effect of IgG on odour-binding, the sample (volume, 1 ml; protein concentration, 1 mg/ml for both $20000 \times g$ pellet and the Triton X-100 extract of $20000 \times g$ pellet) was incubated with [^{3}H]camphor or [^{3}H]decanal ($5 \cdot 10^{-10}$ M) in the presence of IgG* for 1 h at 4°C. Bound ligand was separated from free ligand by centrifugation, for $20000 \times g$ pellet ($15000 \times g$ for 10 min), and by gel filtration (column 0.8–5 cm with Sephadex G-50), for Triton X-100 extract. Non-specific binding was measured in the presence of 1 μM unlabeled ligand. The effect of IgG* was expressed as a ratio of bound ligand in the presence of IgG* to the control.

Electrophysiology. The rats were killed by cervical dislocation, the head was removed and the olfactory turbinates were exposed. The excised head was placed in a humid chamber with a continuous flow of moist clean 95% O_2/5% CO_2 mixture through a pipette directed at the olfactory turbinates, with a flow rate of 20–40 ml/min. The recording electrode (a glass micropipette with tip diameter of 10–30 μm filled with saline) was placed at the surface of the epithelium. The electroolfactogram was recorded through a solid-state amplifier with an input resistance of more than 10^{10} Ω and an input current of less than $5 \cdot 10^{-13}$ A. For stimulation, 0.1 ml of saturated vapour of camphor and decanal mixture was introduced into the carrier stream (the stimuli dilution was 1/30–1/100). Each animal was exposed to 3–5 odour stimuli, with 2 min between each stimulus. The preparations which gave an electroolfactogram amplitude of about 1 mV were selected for the antibody study. In these preparations the electroolfactogram amplitude was invariant for an hour or more. The olfactory epithelium was washed with Ringer solution for 5 min at the flow rate of 50 ml/h, the electroolfactogram was registered, the olfactory epithelium was washed again with Ringer solution for 5 min, and IgG* in Ringer solution (1 mg/ml) was applied directly to the surface of epithelium by micropipette. The volume of the applied antibody was 5–10 μl. The electroolfactogram was registered (i) immediately, (ii) after 10 min antibody treatment, (iii) after 20 min washing with Ringer solution.

Results

Isolation of odour-binding glycoproteins

Fig. 1 represents the general scheme of isolation of odour-binding proteins from rat olfactory epithelium. The results of analytical separation of Triton X-100 extract of the $20000 \times g$ pellet are shown in Figs. 2 and 3. It is noteworthy that the binding of labeled ligand does not depend on the concentration of Triton X-100 in the band after isoelectric focusing (Fig. 3). Therefore, the binding of ligand is mainly determined by the binding to protein, but not by its sorption in the hydrophobic core of the detergent micelle. Thus, the main components capable of [^{3}H]camphor binding are the membrane proteins with the molecular mass of approx. 140 kDa and p*I* 4.9 described before [5,6]. The SDS-gel electrophoresis patterns of isolated proteins visualized with silver staining have revealed two major polypeptides, M_r 88000 and 55000 (Fig. 4). It should be noted that the content of these polypeptides is approx. 0.1% of the total membrane olfactory epithelium protein. The polypeptides M_r 88000 and 55000 bind mannose-specific lectin concanavalin A; they are, therefore, glycoproteins. Later on, they will be designated by gp88 and gp55.

According to the chromatography on Ultrogel AcA-34 in the presence of Triton X-100, the molecular mass of the odour-binding proteins of

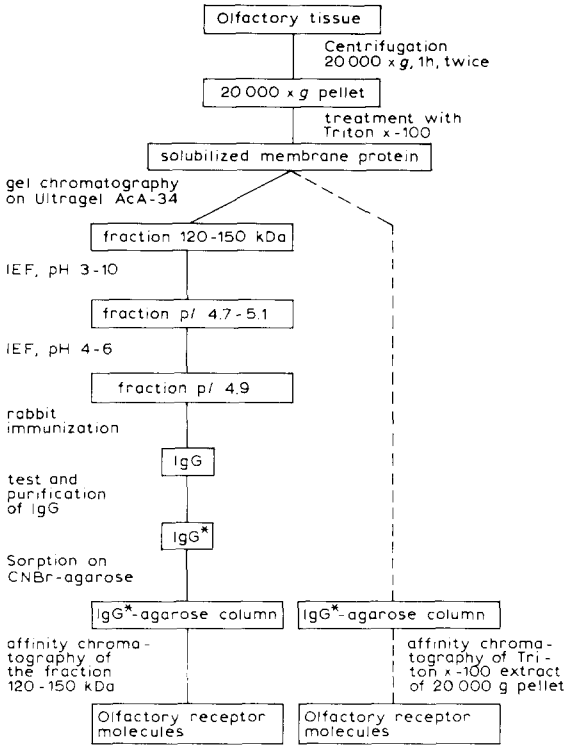

Fig. 1. Scheme of preparative isolation of odour-binding glycoproteins from rat olfactory epithelium.

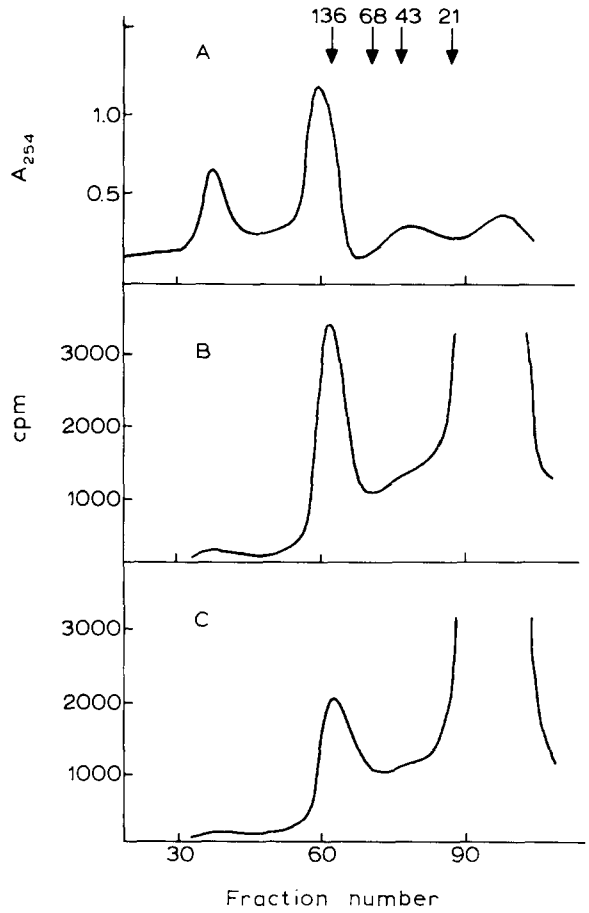

Fig. 2. Analytical gel chromatography of Triton X-100 extract on Ultrogel AcA-34 column. The column (1.6×100 cm) was equilibrated and eluted with 50 mM Tris-HCl (pH 7.2) containing 0.1% Triton X-100 and 0.1 M NaCl. The numbers represent molecular mass markers (kDa). (A) A_{254}, (B) [^{3}H]decanal, (C) [^{3}H]camphor.

olfactory epithelium was approx. 140 kDa, which approximates the sum of the masses of gp88 and gp55. These polypeptides appear to be components of one complex. This is supported by electric focusing in the presence of Triton X-100, when both glycoproteins (gp88 and gp55) are revealed in one band. Therefore, this complex will be designated by gp88/55.

Similar results were obtained when decanal was used as a labeled odorant (the substance with flower odour, Fig. 2). Camphor and decanal do not compete for the binding sites in the rat olfactory epithelium [11]. At the same time, it is unlikely that one and the same protein contains two independent binding sites for different odours. These are evidently different proteins with very similar properties which prevent their separation by the methods described. If the supposition is correct, it is highly probable that gp88/55 also contains the proteins specific to different odours.

Antibodies to gp88/55

The glycoproteins gp88/55 isolated by preparation chromatography on Ultrogel AcA-34 and by preparation focusing in Sephadex, were used as antigens to obtain rabbit antibodies (Table I). The obtained immunoglobulins (IgG) were tested for tissue specificity. The IgGs were found to cross-react strongly with extracts of olfactory epithelium and slightly with those of lung. There were no reactions with extracts of brain, olfactory bulb and liver (Fig. 5). There are two explanations for reaction of IgG with olfactory epithelium and lung. Firstly, this is due to the presence of minor

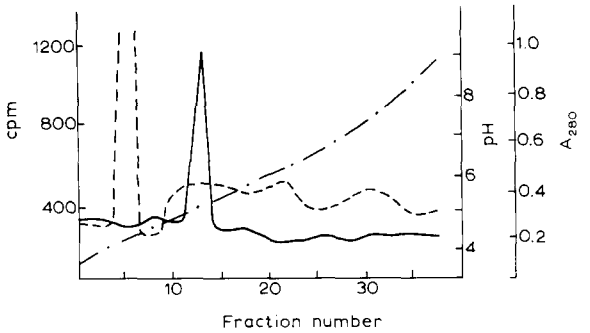

Fig. 3. Isoelectric focusing of M_r 120000–150000 fractions in polyacrylamide gel in the presence of 0.1% Triton X-100. The concentrations of acrylamide, ampholytes and Triton X-100 were 5.0, 1.0 and 0.1%, respectively, pH range, 3–10; ligand, [^{3}H]camphor. ———, radioactivity; — — —, distribution of Triton X-100; ·–·–·, pH.

proteins in gp88/55 of both tissue types. Secondly, this may be explained by the common antigenic determinants of gp88/55 with some

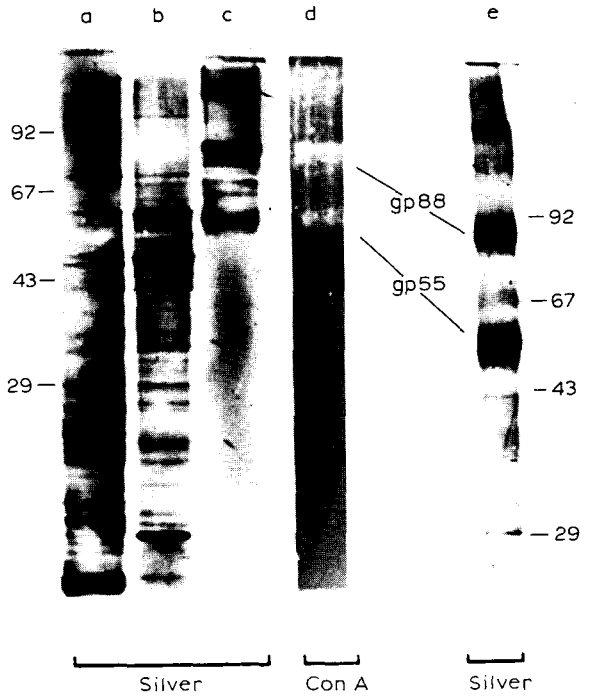

Fig. 4. SDS-gel electrophoresis patterns. (a) Triton X-100 extracts of 20000×g pellet, (b) M_r 120000–150000 fraction from gel chromatography, (c) pI 4.9 fraction from IEF in pH range 4–6, which was used as antigen for immunization, (d) pI 4.9 fraction FITS-labelled concanavalin A staining, (e) gp88/55 purified by IgG*-agarose column. Silver, silver staining; Con A, FITS-labelled concanavalin A staining.

TABLE I

PURIFICATION OF gp88/55

Ligand, [^{3}H]camphor. For binding assay, the sample (1 ml) was incubated with [^{3}H]camphor (10^{-9} M) for 1 h at 4°C. Bound ligand was separated from free ligand by gel filtration (0.8×5 cm column with Sephadex G-50). Nonspecific binding was measured in the presence of μM unlabelled ligand. Protein concentration was determined by the assay described by Bradford [21].

Step	Protein (mg)	Specific binding (pmol/mg)
Triton X-100 extract	700	6
Fraction		
120–150 kDa	90	16
pI 4.7–5.1	3.4	310
PI 4.9	0.45	1100

membrane proteins of lung. (Evidently, the latter supposition is more probable. The gp88/55 isolated by the IgG*-agarose column — see below — also produced antibodies cross-reacting with lung.) To increase the antibody specificity, the cross-reacting polyclonal activity was exhausted

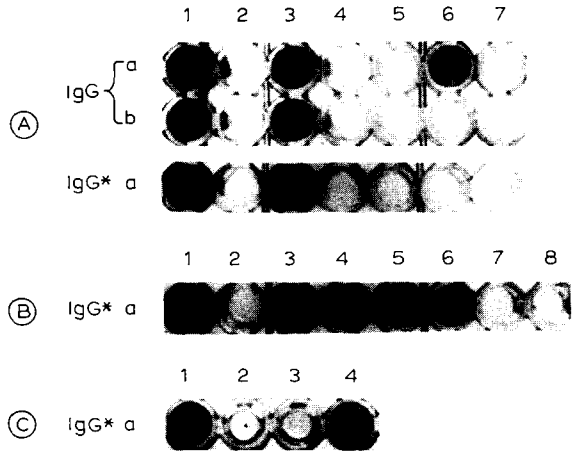

fig. 5. Enzyme immunoassay of IgG and IgG*. (A) TIssue specificity of IgG and IgG*: 1, gp88/55; 2, control; 3, olfactory epithelium; 4, brain; 5, olfactory bulb; 6, lung; 7, liver. (B) Species specificity of IgG*: 1, gp88/55; 2, control; 3, rat; 4, mouse; 5, guinea pig; 6, hamster; 7, frog; 8, carp. (C) IgG* reaction with effluent (3) and eluate (4) of Triton X-100 extract of 20000×g pellet of rat olfactory epithelium after passage through a Con A-Sepharose column. Stock enzyme-antibody conjugate: anti-rabbit IgG-peroxidase conjugate developed in goat. Enzyme substrate: O-phenylenediamine (0.04%) and H_2O_2 (0.012%) in phosphate-citrate buffer (pH 5.0).

with 20000 × *g* pellet of lung, and IgG was assayed again for tissue specificity. The purified immunoglobulins (IgG*) did not cross-react with any type of control tissues, including the lung (Fig. 5). The preliminary immunohistochemical analysis indicated the structures interacting with IgG* to be mainly localized on the surface layer of the epithelium (unpublished data). This means that gp88/55 are mainly localized in the olfactory cilia, knobs and, probably, in the apical end of olfactory cell dendrites.

*Species specificity of IgG**

To elucidate the species specificity of IgG*, an enzyme immunoassay (EIA) of IgG* with extracts of olfactory epithelia of different vertebrate classes was carried out. The analysis has shown the IgGs* to react only with olfactory epithelia of rat, mouse, guinea-pig and hamster but not with those of carp and frog (Figh. 5). Apparently, the immunoglobulins obtained are specific for mammals only.

Effect of the IgG on the rat electroolfactogram*

If the odour-binding glycoproteins are olfactory receptor molecules indeed, it is probable that antibodies raised against these proteins may serve as a potent inhibitor of the rat physiological response of olfactory neurons to odorants. So the effect of IgG* on electroolfactogram was investigated. As IgG* was raised against the mixture of the glycoproteins that bound both camphor and decanal, the olfactory stimulus was a mixture of camphor and decanal.

The mucosa treatment by control IgG decreased the electroolfactogram amplitude to 55–65% of its initial level. However, this decrease was reversible. The mucosa washing with Ringer solution restored the electroolfactogram amplitude up to its initial level (Fig. 6c). At the same time, the IgG* treatment of rat olfactory epithelium decreased the electroolfactogram amplitude to zero. The mucosa washing with Ringer solution restored the electroolfactogram amplitude up to 30% of the initial level, only without changing the form and the duration of the electroolfactogram (Fig. 6d). The response inhibition was observed about 10 min after mucosa treatment by IgG*. This is likely to be due to the low rate of antibody

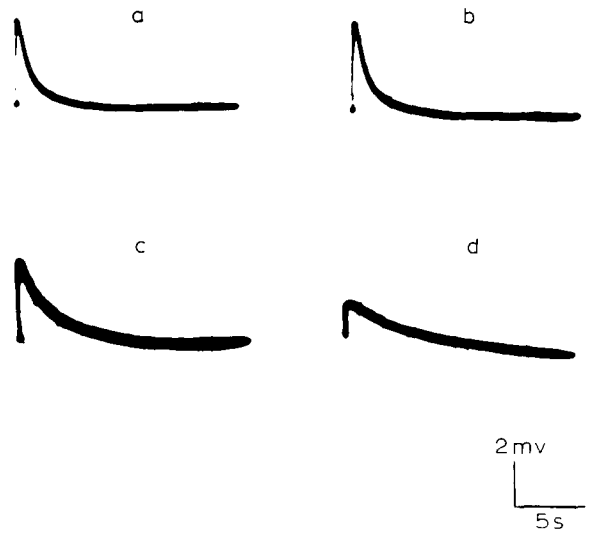

Fig. 6. Effect of IgG* on electroolfactogram from a rat in response to stimulation with a mixture of camphor and decanal. The responses were recorded: (a) Prior to introduction of any fluid on olfactory mucosa, (b) following treatment with saline, (c) following treatment with control IgG and subsequent saline washing for 20 min, (d) following treatment with IgG* and subsequent saline washing for 20 min.

binding to the functionally significant determinants of gp88/55, which resulted from the low concentration of corresponding antibodies and the low association constant rate of IgG* to gp88/55.

Obviously, the reversible inhibition of electroolfactogram by control antibody accounts for the nonspecific sorption of these antibodies on the olfactory epithelium surface. This action is removed by subsequent washing with Ringer solution.

Effect of IgG on binding of odorants by rat olfactory epithelium preparations*

It is unlikely that application of IgG* would allow the antibody to reach intracellular sites or a region separated from the luminal surface of epithelium. Therefore, it is supposed that IgG* reacts only with the protein localized in the apical membranes of olfactory epithelium cells. In this case, the irreversible decrease of the electroolfactogram amplitude suggests the formation of gp88/55-IgG* complex (if gp88/55 is really a receptor) to effect the binding of gp88/55 with odorants. To validate this supposition the effect of IgG* on binding of labeled odorants to 20000 × *g* pellet

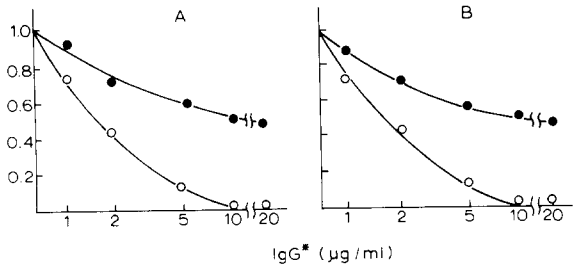

Fig. 7. Effect of IgG* on [^{3}H]camphor and [^{3}H]decanal specific binding to the 20000 × *g* pellet of olfactory epithelium and Triton X-100 extract of 20000 × *g* pellet. The effect of IgG* was expressed as a ratio of bound ligand in the presence of IgG* to the control. (A) [^{3}H]Camphor, (B) [^{3}H]decanal. ●, 20000 × *g* pellet; ○, Triton X-100 extract.

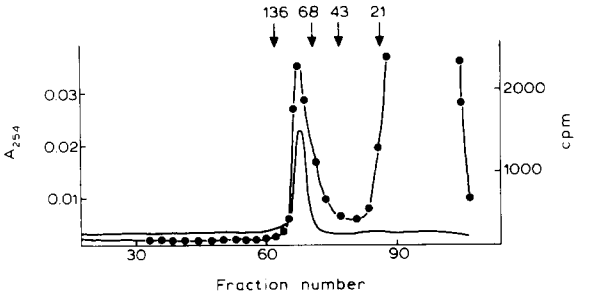

Fig. 8. Gel chromatography of gp88/55 purified by IgG*-agarose column. Chromatography was performed as in Fig. 2. Ligand, [^{3}H]camphor. ———, A_{254}; ●, radioactivity. The numbers represent molecular mass marker (kDa).

and solubilized proteins of this fraction was investigated (Fig. 7). IgG* completely inhibited the specific binding of [^{3}H]camphor and [^{3}H]decanal with Triton X-100 extract of 20000 × *g* pellet and reduced the binding with 20000 × *g* pellet by 50%. Such a diversity can be explained by the different accessibility of IgG* to gp88/55 in both types of preparation. It can be due to the fact that a part of membranes in 20000 × *g* pellet was turned inside out. If gp88/55 was localized on the surface layer of membrane (as it could be in the case of receptor), a part of gp88/55 should be inaccessible to IgG*.

Properties of g88/55

Further on, IgG* was used as immunosorbent to isolate the glycoproteins gp88/55. Besides its simplicity, this method provides an increased purity of the glycoproteins isolated due to the high tissue specificity of antibodies, as shown above. The dissociation of IgG*-antigen complex took place at low pH, so the proteins bound to IgG* column were eluted at pH 2.8.

SDS-gel electrophoresis of the proteins bound to IgG*-agarose column revealed two major polypeptides, gp88 and gp55, (Fig. 4). These data support the IgG* effect on ligand binding and on electroolfactogram to be related to the IgG* effect on gp88/55 only.

The proteins bound to the IgG* column were eluted at pH 2.8. The low pH of the eluate might result in partial denaturation of the proteins and, therefore, in loss of ability to bind [^{3}H]camphor and [^{3}H]decanal. Nevertheless, the component capable of binding a labeled ligand was isolated after gel chromatography of this gp88/55 on Ultrogel AcA-34 in the presence of Triton X-100 (Fig. 8). However, the results obtained were unexpected. According to gel chromatography, the molecular mass of the binding component was lower than that determined by gel chromatography of the Triton X-100 extract of the 20000 × *g* pellet. SDS-gel electrophoresis patterns of the fraction of gel chromatography show the molecular mass of the component capable of binding ligand to be 88 kDa (Fig. 9). Another component (gp55) appears to be not capable of binding odorants.

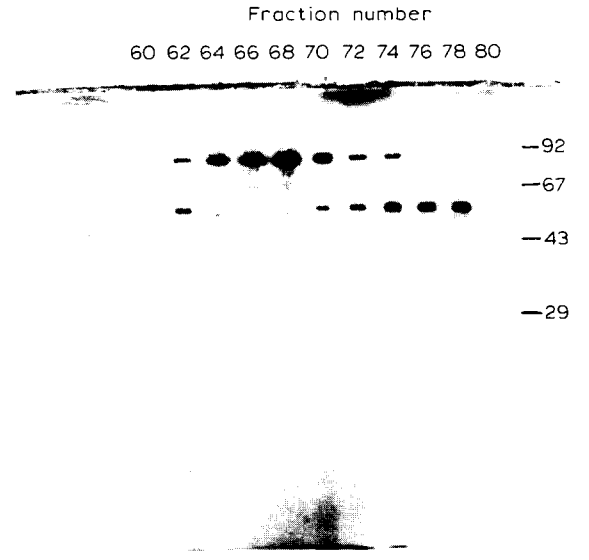

Fig. 9. SDS-gel electrophoresis patterns of the fraction from gel chromatography in Fig. 8.

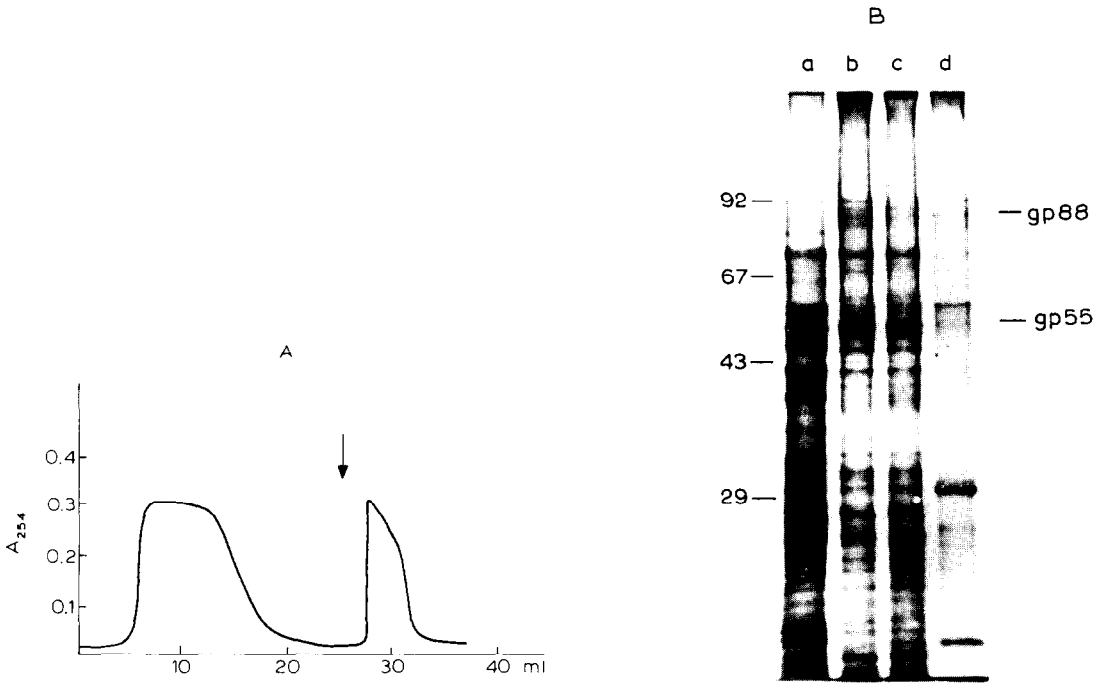

Fig. 10. (A) Affinity chromatography of the M_r 120000–150000 fraction on Con A-agarose. The column (0.6×2.5 cm) was equilibrated and eluted with 50 mM Tris-HCl (pH 7.0) containing 0.1% Triton X-100 and 0.1 M NaCl. The arrow points to addition of 1-*O*-methyl-α-D-mannopyranoside (50 mg/ml). (B) SDS-gel electrophoresis patterns: (a) Triton X-100 extract, (b) M_r 120000–150000 fraction, (c) effluent from the column, (d) eluate from the column. Silver staining.

Thus, the data obtained suggest that the ligand-binding component from rat olfactory epithelium is the glycoprotein with M_r approx. 140000 consisting of two subunits (gp88 and gp55), gp88 being capable of odour-binding. This complex is stable in the presence of Triton X-100 at physiological pH values during gel exclusion chromatography and isoelectric focusing. At the same time the gp88/55 complex dissociates at acid pH value (for example, during affinity chromatography at pH 2.8).

Partial purification of odour-binding proteins by lectins

The gp88/55 complex interacts with concanavalin A, as shown above. Therefore, a Con A-Sepharose column was used for partial purification of gp88/55. The M_r 120000–150000 fraction of the 20000 × *g* pellet was chromatographed on the given sorbent. The proteins bound to Con A-Sepharose made up less than 10% of all the proteins of a Triton X-100 extract of the 20000 × *g* pellet (Fig. 10). The enzyme immunoassay demonstrated that only the proteins binding to Con A-Sepharose were able to interact with IgG* (Fig. 5). Thus, this sorbent can be effectively used on the partial purification of isolating gp88/55.

To ascertain the turnover of gp88/55 in the olfactory epithelium, the isolated mucosa was incubated with [^{3}H]leucine. The uptake of [^{3}H]leucine was determined in gp88/55 isolated with IgG*-agarose column, in total cytoplasmic and membrane proteins (Fig. 11). The [^{3}H]leucine uptake into gp88/55 is 4.5- and 1.5-times higher than that into membrane proteins and cytoplasma proteins, respectively. Thus, the turnover of odour-binding glycoproteins from olfactory epithelium is relatively high.

Discussion

The evidence presented here demonstrates that the glycoproteins gp88/55 isolated from rat olfactory epithelium possess a number of properties

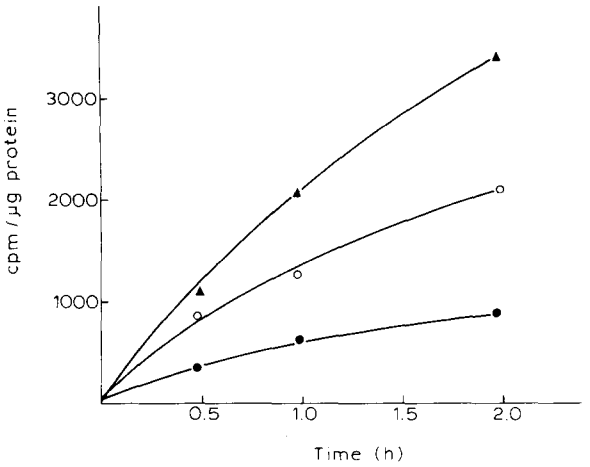

Fig. 11. Time course of the appearance of [^{3}H]-labelled gp88/55 in the olfactory epithelium. 15 isolated olfactory epithelia were incubated at 37°C in Ringer solution containing 1 mCi [^{3}H]leucine. After the appropriative incubation time, five olfactory epithelia were removed and washed twice with Ringer solution. The tissues were immediately homogenized in 10 mM Tris-HCl buffer (pH 7.2) containing 0.1 M NaCl, and then centrifuged at 20000 × *g* for 20 min. 20000 × *g* pellets were solubilized with 0.3% Triton X-100, Triton X-100-soluble protein was applied on an IgG*-agarose column and the radioactivity of the protein bound to column was measured. ●, total membrane protein. ○, total soluble protein; ▲, gp88/55.

necessary for relating the proteins investigated to receptor molecules. Firstly, they are able to bind odorants effectively. Secondly, they are tissue-specific transmembrane proteins which are present in olfactory epithelium only. Moreover, the preliminary studies have shown the glycoproteins gp88/55 to be localized in the surface layer of olfactory epithelium, where the olfactory receptor structures are supposed to be localized. Finally, the antibodies to the glycoproteins isolated inhibit the binding of odorants, this inhibition being of a functional character: the physiological response of olfactory receptor cell to an odorant is inhibited simultaneously. Thus, the glycoproteins from olfactory epithelium investigated are plausible candidates for olfactory receptor molecules.

The gp88/55 glycoproteins essentially differs from anisole-binding protein isolated by Price [1]. The latter is a water-soluble protein; therefore, it does not seem to be a receptor molecule. At the same time, antibodies raised against anisole-binding protein specifically inhibited the electroolfactogram [15]. This indicates that anisole-binding protein seems to participate in olfactory reception.

The specific glycoproteins were found by Lancet's group in frog (gp95 and gp58) and rat (gp85 and gp55) olfactory cilia [9,10,16]. Proceeding from some properties of them, the authors consider these glycoproteins (especially gp95) to be the most plausible candidates for olfactory receptor molecules. Unfortunately, there is as yet no evidence indicating the above glycoproteins to be functionally identical to the gp88/55 described in the present paper. This problem needs to be investigated.

Binding activity was demonstrated for the subunit gp88 only, but was not found for gp55. Further investigations are required to determine whether gp88/55 complex or only subunit gp88 is the olfactory receptor. The relative stability of gp88/55 complex demonstrated by gel chromatography and isoelectric focusing suggests it to be a receptor candidate. (Thus, if the function of gp88 is clear, that of gp55 is unknown.) Probably, gp55 participates in the mechanism of signal transduction. Supplementary investigations are required to solve this problem. On the other hand, the possibility should not be excluded that the formation of relatively stable complex gp88/55 is an artefact of isolation, although such a probability is not high, primarily because of insufficient content of gp88 and gp55 in olfactory epithelium.

Evidently, gp88 is heterogeneous and represents a set of polypeptides with similar properties. The differences between them are mainly determined by the structure of the sites recognizing odorants. This does not allow the separation of these glycoproteins by methods applied in the present work. In our opinion, there are two methods of separating specific glycoproteins. The first method is to obtain monoclonal antibodies raised against different distinguishing odorant centres of these peptides. This procedure is extremely difficult. Another method of isolating specific gp88 is to use the sorbents immobilized by certain classes of odorants [1]. This method is especially convenient for fish, because amino acids are effective stimuli for them [20,21] and can be easily used as modificators. Using this method, a number of proteins

which bind amino acids were isolated from the skate olfactory epithelium, as follows: 'alanine-serine', 'methionine', 'lysine' and 'glutamate' binding protein. Previous experiments demonstrated that they all are membrane glycoproteins consisting of two subunits (gp98 and gp56), the large one (gp98) being capable of binding amino acids.

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