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The subunits of specific odor-binding glycoproteins from rat olfactory epithelium

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The specific odor-binding glycoproteins have been isolated from rat olfactory epithelium. They consist of two subunits, gp88 and gp55. Subunit gp88 is capable of odorant binding.

Odor; Olfactory epithelium; Receptor; Glycoprotein

1. INTRODUCTION

Odorant binding with the receptor molecules of olfactory sensory neurons is universally believed to be the first step in vertebrate chemoreception. However, the molecular identity of such molecules has not been clearly established. Using binding assays or isolation of specific proteins of olfactory cilia, a number of candidates for receptor molecules have been suggested but satisfactory identification has not been achieved [1–9].

It is clear that only simultaneous use of the main criteria of the receptor would allow identification of the olfactory receptor molecules [10]. Recently, using gel chromatography and isoelectric focusing, we isolated membrane proteins from rat olfactory epithelium possessing a high affinity with two non-competing odors, camphor and decanal [11]. These proteins have a very similar molecular mass (~140 kDa) and isoelectric point (p*I* 4.9). Apparently, they constitute a group of odor-binding proteins from olfactory epithelium which have very similar properties. These proteins were revealed to be tissue-specific to olfactory epithelium only. They also exhibit species specificity, being present in mammals but not in fish and amphibians.

Rabbit antibodies have been raised against odor-binding proteins [11]. These antibodies block the binding of olfactory epithelium preparations with both camphor and decanal and irreversibly inhibit the physiological response of the olfactory mucosa to both odorants (EOG).

The properties enumerated suggest that the isolated odor-binding proteins could be plausible candidates for olfactory receptor molecules. Here, the properties of odor-binding proteins from rat olfactory epithelium are investigated.

2. MATERIALS AND METHODS

2.1. *Antibodies*

Rabbit antibodies were raised against odor-binding proteins from rat olfactory epithelium as described [11]. The serum titer was 1/6000 as determined by ELISA using anti-rabbit IgG peroxidase conjugate developed in goat. IgG fraction was obtained using a protein A-Sepharose affinity column.

2.2. *Affinity chromatography*

IgG was coupled to CNBr-activated agarose by standard methods and an IgG-agarose affinity col-

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umn used for purification of odor-binding protein. A fraction of 120–150 kDa of a Triton X-100 extract of the 20000 × *g* pellet of rat olfactory epithelium [11] was applied to the IgG-agarose column, which was equilibrated with 50 mM Tris-HCl buffer containing 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.

2.3. *Gel chromatography*

An Ultrogel AcA-34 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 radioactivity ([^{3}H]camphor or [^{3}H]decanal, 10^{-9} M) was added to samples before chromatography.

2.4. *Electrophoresis*

Electrophoresis in 10% polyacrylamide gels in the presence of SDS and 2-mercaptoethanol was carried out using a discontinuous buffer system according to Laemmli [12]. Polypeptides were visualized by silver staining [13]. Glycoproteins were identified directly on the electrophoresis gel by the lectin overlay method [14] with fluoresceinated concanavalin A (Con A).

3. RESULTS

Fig.1 depicts SDS gel electrophoretic patterns of odor-binding proteins from rat olfactory epithelium isolated using the IgG-agarose column. Silver staining revealed two major bands – 88 and 55 kDa. Fig.1 also shows the glycoprotein patterns visualized by binding of the mannose-specific lectin Con A to the electrophoretic gel. The patterns demonstrate that these two major bands visualized with silver are glycoproteins (gp88 and gp55).

The molecular mass of odor-binding proteins is ~140 kDa (see section 1) which approximates to the sum of the masses of gp88 and gp55 revealed after SDS gel electrophoresis. Most probably, these polypeptides are the subunits of the same glycoproteins (140 kDa). They will henceforth be designated gp88/55.

The bound proteins to the IgG column were eluted at low pH (pH 2.8) which might result in their partial denaturation, being responsible for the inability to bind [^{3}H]camphor and

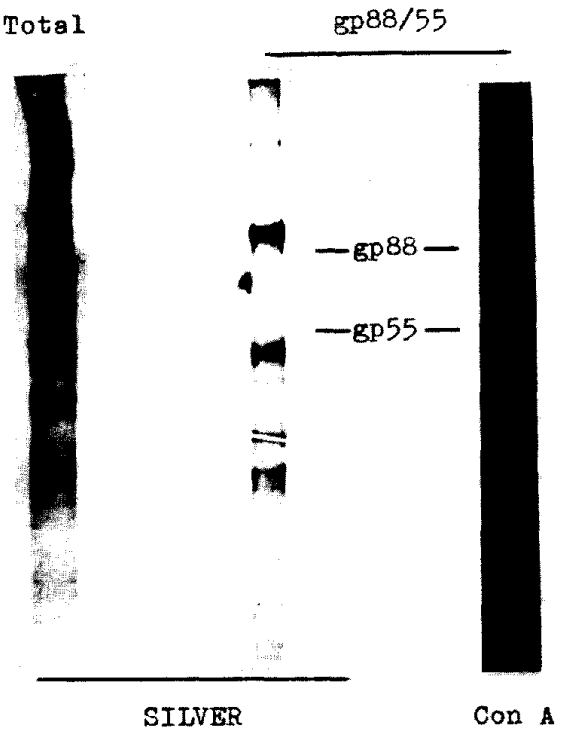

Fig.1. SDS gel electrophoretic pattern. Total, Triton X-100 extract of the 20000 × *g* pellet; gp88/55, purified odor-binding glycoprotein; SILVER, silver staining; Con A, binding of fluorescein-labeled concanavalin A to gp88/55.

[^{3}H]decanal. Nevertheless, the component capable of binding ligand was isolated after gel chromatography of these proteins on Ultrogel AcA-34 in the presence of Triton X-100 (fig.2). However, the result obtained was unexpected. Ac-

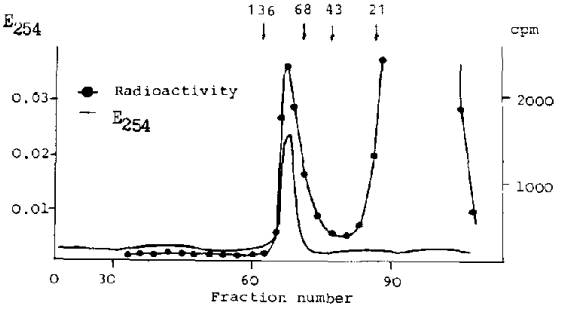

Fig.2. Gel chromatography of gp88/55 on an Ultrogel AcA-34 column. (——) E_{254}; (●——●) radioactivity.

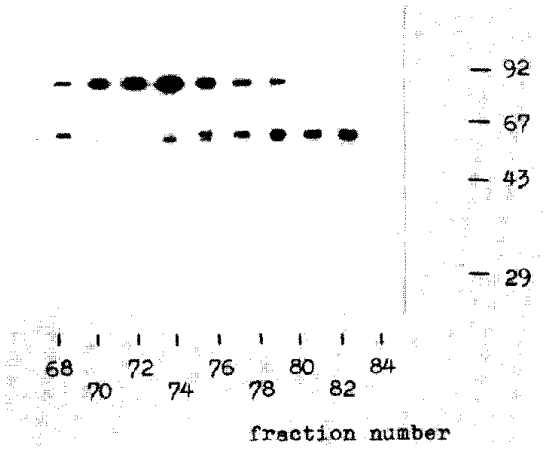

Fig.3. SDS gel electrophoretic patterns of the fraction from gel chromatography.

cording to chromatography on Ultrogel AcA-34, the molecular mass of the odor-binding component was lower (~90 kDa), compared to that of odor-binding protein that had been isolated previously (~140 kDa) [11]. In fig.2 this is indicated by the arrow. Fig.3 depicts the SDS gel electrophoretic patterns of the fractions shown on the chromatogram. As shown in fig.3, the fractions able to bind ligands contained subunit gp88 only. Probably, gp55 does not bind stimuli. The data obtained suggest that the gp88/55 complex obviously dissociates at acidic pH values during affinity chromatography at pH 2.8. It is therefore supposed that ionic forces contribute to the formation of the gp88/55 complex.

4. DISCUSSION

As has been indicated, glycoprotein gp88/55 isolated from rat olfactory epithelium exhibits a number of the properties of olfactory receptor molecules. Glycoproteins with similar properties have been found by Lancet's group in frog (gp55 and gp58) and rat (gp85 and gp55) olfactory cilia [9]. According to some of the properties, they consider glycoproteins (especially gp95) to be the most probable candidates for receptor molecules. These glycoproteins are likely to be functionally identical to gp88/55 from rat. If this is really so, then all of them may represent the class of olfactory receptor molecules.

Binding activity was demonstrated in the gp88 subunit but was not found in gp55. Further work is required to determine whether the gp88/55 complex or only the gp88 subunit is the olfactory receptor. The relative stability of the gp88/55 complex, demonstrated by gel chromatography and isoelectric focusing, suggests it to be a receptor. (Thus, if the function of gp88 is clear, then that of gp55 is unknown.) On the other hand, the possibility cannot be excluded that the formation of the relatively stable complex gp88/55 is an artefact of isolation. However, in our opinion, this is very unlikely, most important of all, because of their content being insufficient in the olfactory epithelium.

REFERENCES

[1] Cagan, R.H. and Zeiger, W.N. (1978) Proc. Natl. Acad. Sci. USA 75, 4679–4683.
[2] Price, S. (1978) Chem. Sens. Flavor 3, 51–56.
[3] Fesenko, E.E., Novoselov, V.I. and Krapivinskaja, L.D. (1979) Biochim. Biophys. Acta 587, 424–433.
[4] Fesenko, E.E., Novoselov, V.I., Krapivinskaja, L.D., Mjasoedov, N.F. and Zolotarev, J.A. (1983) Biochim. Biophys. Acta 759, 250–256.
[5] Persaud, K.C., Wood, P.H., Squirrel, D.J. and Dodd, G.H. (1981) Biochim. Soc. Trans. 9, 107–108.
[6] Rhein, L.D. and Cagan, R.H. (1983) J. Neurochem. 41, 569–577.
[7] Bignetti, E., Cavaggioni, A., Pelosi, P., Persaud, K.C., Sorbi, R.T. and Tirindelli, R. (1985) Eur. J. Biochem. 149, 227–231.
[8] Chen, Z. and Lancet, D. (1984) Proc. Natl. Acad. Sci. USA 81, 1859–1863.
[9] Chen, Z., Pace, U., Ronen, D. and Lancet, D. (1986) J. Biol. Chem. 261, 1299–1305.
[10] Lancet, D. (1986) Annu. Rev. Neurosci. 9, 329–356.
[11] Novoselov, V.I., Bystrova, M.F. and Fesenko, E.E. (1987) Sensory Syst. 1, 7–12 (in Russian).
[12] Laemmli, U.K. (1970) Nature 227, 680–685.
[13] Oakley, B.R., Kirsh, D.R. and Morris, N.R. (1980) Anal. Biochem. 105, 361–366.
[14] Burridge, K. (1978) Methods Enzymol. 50, 54–64.