45-kDa GTP-binding Protein from Rat Olfactory Epithelium: Purification, Characterization and Localization

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Abstract

The rat olfactory epithelium contains a specific water-soluble 45-kDa protein. This protein is recognized by anti-peptide antibodies which react with α-subunits of the known G-proteins. The 45-kDa protein has been isolated using DEAE-chromatography and gel-exclusion chromatography. The content of 45-kDa protein is about 2% of the total soluble proteins of the olfactory mucosa and it is located at the mucociliary surface. According to photo-affinity labeling, the 45-kDa protein possesses a high affinity to GTP and exhibits low GTP hydrolytic activity. The functions of the 45-kDa protein are discussed. **Chem. Senses 21: 181–188, 1996.**

Introduction

It is generally accepted that odorant recognition and olfactory transduction are initiated at the mucociliary surface of the olfactory mucosa. This area of the olfactory mucosa includes mucus, knobs of olfactory cells, cilia of olfactory cells and microvilli of sustentacular cells. Different odorants activate distinct receptor cells that encode odor quality and quantity. Two independent second messenger cascades have been found in olfactory neurons each being specific for distinct odorant classes (Breer *et al.*, 1990). Different G-proteins suggested to be involved in these cascades have been identified (Jones and Reed, 1989; Jones *et al.*, 1990). Recently, a novel multi-gene family of G-protein-linked receptors that are expressed uniquely in olfactory epithelium have been discovered (Buck and Axel, 1991; Ngai *et al.*, 1993a,b; Ramming *et al.*, 1993; Ressler *et al.*, 1993; Krieger *et al.*, 1994). Olfactory mucosa also contains several types of odor-binding proteins, including both membrane and water-soluble proteins (Bignetti *et al.*, 1985; Fesenko *et al.*, 1988; Pevsner *et al.*, 1988; Novoselov *et al.*, 1988, 1989). Finally, some specific membrane proteins, unique to chemosensory cilia have been found (Chen and Lancet, 1984; Chen *et al.*, 1986; Anholt *et al.*, 1990; Snyder *et al.*, 1991).

To study the possible mechanisms of signal transduction, we tried to reveal different G-proteins in olfactory epithelium. We produced sequence-specific antibodies raised against the guanosine 5′-triphosphate (GTP)-binding region of the α-subunits of G-proteins. This region is common for all known G-proteins; moreover, some other GTP-binding proteins have the same sequence motif (Spiegel, 1988). Using these antibodies we have found a specific 45-kDa water-soluble GTP-binding protein which has properties different from those of the known G-proteins which have already been identified in rat olfactory epithelium (Novoselov *et al.*, 1994). Our data suggest that 45-kDa protein is not a G-protein and seems to be involved in perireceptor events. This protein is located at the mucociliary surface, most

probably in the mucus layer. No analogous proteins were found in other rat tissues, but a 45-kDa protein was detected in olfactory mucosa of other mammals. In the present paper, we describe some of the properties of this 45-kDa GTP-binding protein.

Materials and methods

Materials

[α-^{32}P]GTP, [γ-^{32}P]GTP (>2000 Ci/mmol; from Physics and Energy Institute, Obninsk, Russia); rabbit Anti-Actin (A 2066) from Sigma (St Louis, USA); peroxidase-conjugated goat anti-rabbit Ig from Amersham, UK; Dulbecco's modified Eagle's Medium (DME), GTP, adenosine 5′-triphosphate (ATP), Triton X-100, 3′,3-diaminobenzidine, chemicals for electrophoresis from Sigma (St Louis, USA); DEAE-Sepharose, Sephacryl S-200 from Pharmacia (Uppsala, Sweden); Immobilon PVDF from Millipore (Bedford, USA).

Preparation of anti-peptide antisera

The sequence of a synthetic peptide for the generation of antisera against α_{common} (CGAGESGKSTIVKQMK), the peptide sequence found in α-subunit of all known G-proteins was deduced from published sequences of cDNAs encoding the α-subunits of G-proteins (Mumby *et al.*, 1986). Peptide synthesis and coupling to keyhole limpet hemocyanin were performed as described before (Green *et al.*, 1982).

Female New Zealand white rabbits were used for the generation of the antisera against α_{common} and the 45-kDa kDa-protein. For the generation of antisera against α_{common} 100 μg of cross-linked peptide in complete Freund's adjuvant (total volume of 1.5 ml) were injected by intradermal injections in the back. The immunization procedure was repeated 2 weeks later with the same amount of antigen emulsified in incomplete Freund's adjuvant. Antiserum was obtained 4 weeks after first injection. Titer of antisera was monitored by ELISA, as described previously (Mumby *et al.*, 1986). For generating antiserum against 45-kDa protein 200 μg of the protein in saline were emulsified in complete Freund's adjuvant (total volume of 1.5 ml). The emulsion was injected into each hind foot pat of the rabbit. After 4 weeks the animal was given a booster injection of 200 μg protein using incomplete adjuvant. After 10 days the animal was bled from ear vein. The serum titer was monitored by enzyme-linked immunosorbant assay (ELISA).

Isolation of 45-kDa protein

Male Wistar rats were killed by decapitation. Nasal turbinates were dissected, pooled and washed in Dulbecco's modified Eagle's Medium (DME), pH 8.0, at 4°C. Tissue was homogenized in the same medium, containing 10 mM $CaCl_2$ (1 ml per mucosa). The homogenate was centrifuged at 20,000 *g* for 1 h, the supernatant was dialysed overnight against buffer consisting 12 mM Tris−HCl at pH 7.8, 1 mM $MgCl_2$, 1 mM dithiothreitol.

The dialysed extract was applied to a DEAE-Sepharose column (12.5 × 305 mm) equilibrated with buffer consisting of 12 mM Tris−HCl at pH 7.8, 1 mM $MgCl_2$, 1 mM dithiothreitol. The column was eluted at 17°C with a 750-ml gradient of 0–500 mM NaCl in the same buffer. Fractions were collected and assayed by immunoblotting using antibodies against α_{common}. DEAE fractions containing immunoreactive 45-kDa protein were concentrated by ultrafiltration with a PM-10 filter (Amicon) and applied directly to a Sephacryl S-200 column (16 × 820 mm) equilibrated with buffer, consisting 25 mM Tris−HCl at pH 7.8, 100 mM NaCl, 1 mM $MgCl_2$, 1 mM dithiothreitol. Fractions were collected and assayed by immunoblotting, using antibodies against α_{common}.

Purified 45-kDa protein was concentrated by ultrafiltration with a PM-10 filter (Amicon) and used for further studies.

Gel electrophoresis and immunoblotting

SDS−polyacrilamide gel electrophoresis was performed after the treatment of the samples with 2-mercaptoethanol on 10% slab gels in discontinuous buffer system of Laemmli (Laemmli, 1970). For immunoblotting the electrophoretic transfer onto an Immobilon PVDF membrane was performed in a 2051 Midget Multiblot Transphor Unit (LKB, Sweden) in 100 mM Tris, 100 mM boric acid, pH 8.2–8.3, for 1 h at 10°C. The incubation with the primary antibody (rabbit antiserum against 45-kDa protein or α_{common}) at appropriate dilution was in 10 mM sodium phosphate buffer, 0.9% NaCl and 0.05% Tween-20 for 3 h at 4°C. The bound antibody was visualized by the formation of complex with rabbit Ig horseradish peroxidase-linked antibody. The complex was visualized in 10 mM sodium phosphate buffer and 0.15 M NaCl, pH 7.5, containing 0.5 mg/ml 3′3-diaminobenzidine tetrachloride and 0.015% hydrogen peroxide.

Analytical electrofocusing was performed in thin-layer polyacrilamide gel containing 2% ampholine (LKB, Sweden).

Light microscopic immunohistochemistry

Immunohistochemical localization of the 45-kDa protein was performed on 6-μm thick sections of formalin-fixed rat olfactory epithelium embedded in paraffin and mounted on chromalum-gelatin-coated microscope slides. The sections were deparaffinized in xylene and rehydrated through a series of graded alcohols from absolute to water. The rehydrated sections were incubated with anti-45-kDa protein rabbit antibodies at 500-fold dilution of the antiserum for 1 h at ambient temperature. The bound antibody was visualized by formation of complex with goat anti-rabbit Ig horseradish peroxidase-linked antibody. The complexes was visualized in 10 mM sodium phosphate buffer and 0.9% NaCl, pH 7.5, containing 0.5 mg/ml 3'3-diaminobenzidine tetrachloride and 0.015% hydrogen peroxide. To evaluate non-specific staining controls were incubated with pre-immune serum. The stained sections were observed under a camera-equipped Zeiss-Eiena microscope.

Photo-affinity labeling

Direct photo-affinity labeling of protein with [^{32}P]-α-GTP was carried out according to Antonoff *et al.* (1976). Protein samples were irradiated with the source having a peak emission at 253.7 nm. Affinity-labeled proteins were analysed by SDS-gel electrophoresis. Gels were auto-radiographed using standard procedure. Kodak X-OMAT XAR-films were used.

GTPase activity was determined according to Baehr *et al.* (1982).

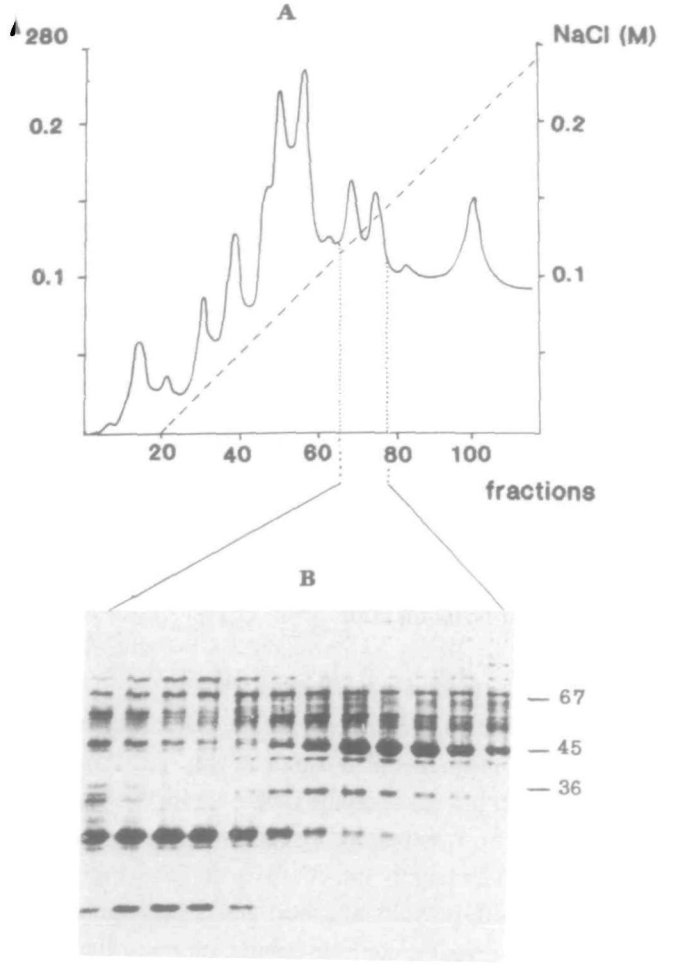

Figure 1 (A) DEAE ion-exchange chromatography on DEAE-sepharose of the water-soluble extract of rat olfactory mucosa. (B) SDS-electrophoresis patterns of indicated fractions after DEAE ion-exchange chromatography. Coomassie R 250 staining.

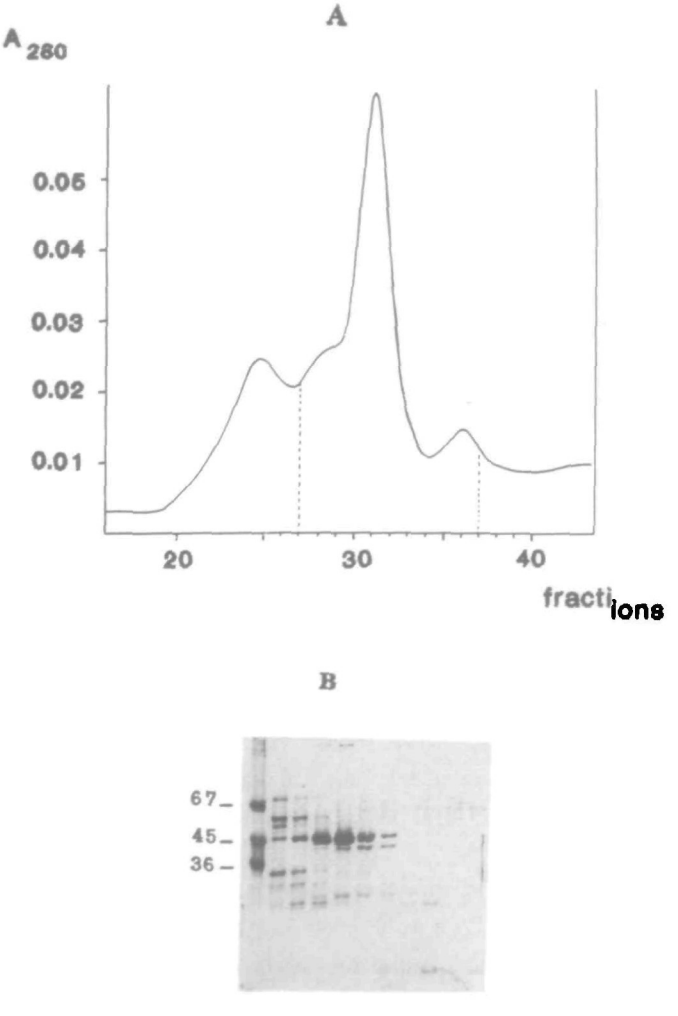

Figure 2 (A) Chromatography on Sephacryl S-200 column of the fraction obtained by DEAE ion-exchange chromatography. (B) SDS-electrophoresis patterns of indicated fractions after chromatography of Sephacryl S-200 column. Coomassie R 250 staining The 45-kDa protein was isolated as a single polypeptide with molecular mass about 45 kDa.

Protein concentrations were measured according to the method of Bradford (1976) using bovine serum albumin as standard.

Results

Isolation of 45-kDa protein

The DEAE-chromatography was used for the first step of the 45-kDa protein isolation. Figure 1 shows chromatography of the water-soluble extract of rat olfactory epithelium and SDS-polyacrilamide gel electrophoresis patterns of the column fractions. Using the DEAE-chromatography, the 45-kDa protein was efficiently separated from other proteins.

For further purification, the fractions containing the 45-kDa protein were collected and subjected to chromatography on Sephacryl S-200 column, and the 45-kDa protein was isolated as a single polypeptide (Figure 2). An approximate molecular mass of the native 45-kDa protein determined by gel-exclusion chromatography was also about 45 kDa. So this protein exists as a monomer in the extract of rat olfactory epithelium.

The iso-electric point of the 45-kDa protein determined by iso-electric focusing in polyacrilamide gel was 5.25.

Using DEAE-chromatography and gel-exclusion chromatography, we have obtained about 15–20 μg of the purified 45-kDa protein from one rat mucosa. Taking into account the protein loss during its isolation and the localization of the 45-kDa protein in the ciliary surface (see below), the local concentration of this protein is higher. An approximate estimation has shown that the content of 45-kDa protein is about 2% of the total water-soluble proteins. So the 45-kDa protein is one of the main water-soluble proteins of the rat olfactory epithelium.

45-kDa protein is not actin

The 45-kDa protein has the molecular mass similar to that of actin (Fulton, 1984). Moreover, actin was found in the olfactory mucosa (Chen and Lancet, 1984). Therefore, to ensure that the 45-kDa protein is not actin, we used antibodies against actin.

Figure 3c presents immunoblotting of different extracts and purified 45-kDa protein with anti-actin antibodies. The antibodies revealed actin not only in water-soluble extracts, but also in membranes of rat olfactory mucosa that is consistent with the finding of Chen and Lancet (1984). On the other hand, the antibodies did not react with the purified 45-kDa protein. So it is unlikely that the 45-kDa protein is actin.

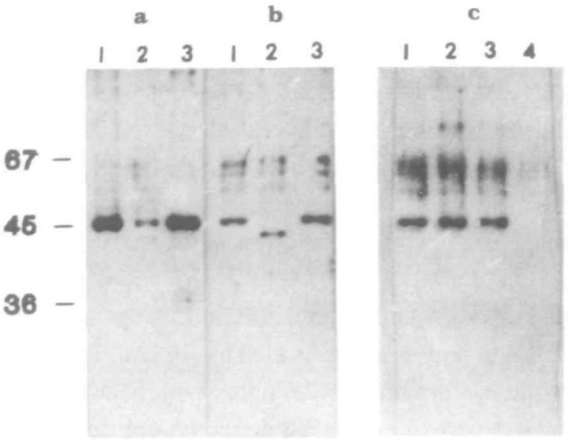

Figure 3 Immunoblots of 45-kDa protein and various extracts from rat olfactory epithelium. (a) Visualization with rabbit antisera against 45-kDa protein: (1) water-soluble extract of rat olfactory mucosa, (2) membrane of rat olfactory mucosa; (3) purified 45-kDa protein. (b) Visualization with rabbit antisera against α_{common} peptide (1) water-soluble extract of rat olfactory mucosa; (2) membrane of rat olfactory mucosa, (3) purified 45-kDa protein. (c) Visualization with rabbit antisera against actin: (1) water-soluble extract of rat olfactory mucosa; (2) membrane of rat olfactory mucosa; (3) water-soluble extract of rat brain, (4) purified 45-kDa protein.

Antibodies against 45-kDa protein

To identifiy 45-kDa protein in the olfactory epithelium, we used the antibodies against α_{common}. In our subsequent study, we used the rabbit antibodies raised against the purified native 45-kDa protein. Figure 3a and b presents immunoblotting of the purified 45-kDa protein, membrane and water-soluble extract of rat olfactory epithelium with both antibodies. The antibodies against 45-kDa protein recognized only 45-kDa protein, but not G-proteins. So, our rabbit antibodies against 45-kDa protein were generated to the determinants which did not include the α-common sequence. Moreover, these determinants were absent in G-proteins of the rat olfactory epithelium.

Localization of 45-kDa protein

To visualize the 45-kDa protein in the olfactory neuroepithelium, immunohistochemical studies were performed at the light microscopic level using rabbit antibodies against the 45-kDa protein. We obtained coronal sections through the rat nasal cavity with largely intact ciliary surface (Figure 4A,B).

Staining with polyclonal rabbit antiserum against the 45-kDa protein reveals intense staining of the ciliary surface and apical part of the receptor, as well as sustentacular cells (Figure 4C,D). Moreover, 45-kDa protein was also found in the area of olfactory epithelium where respiratory cells were predominant (data not shown).

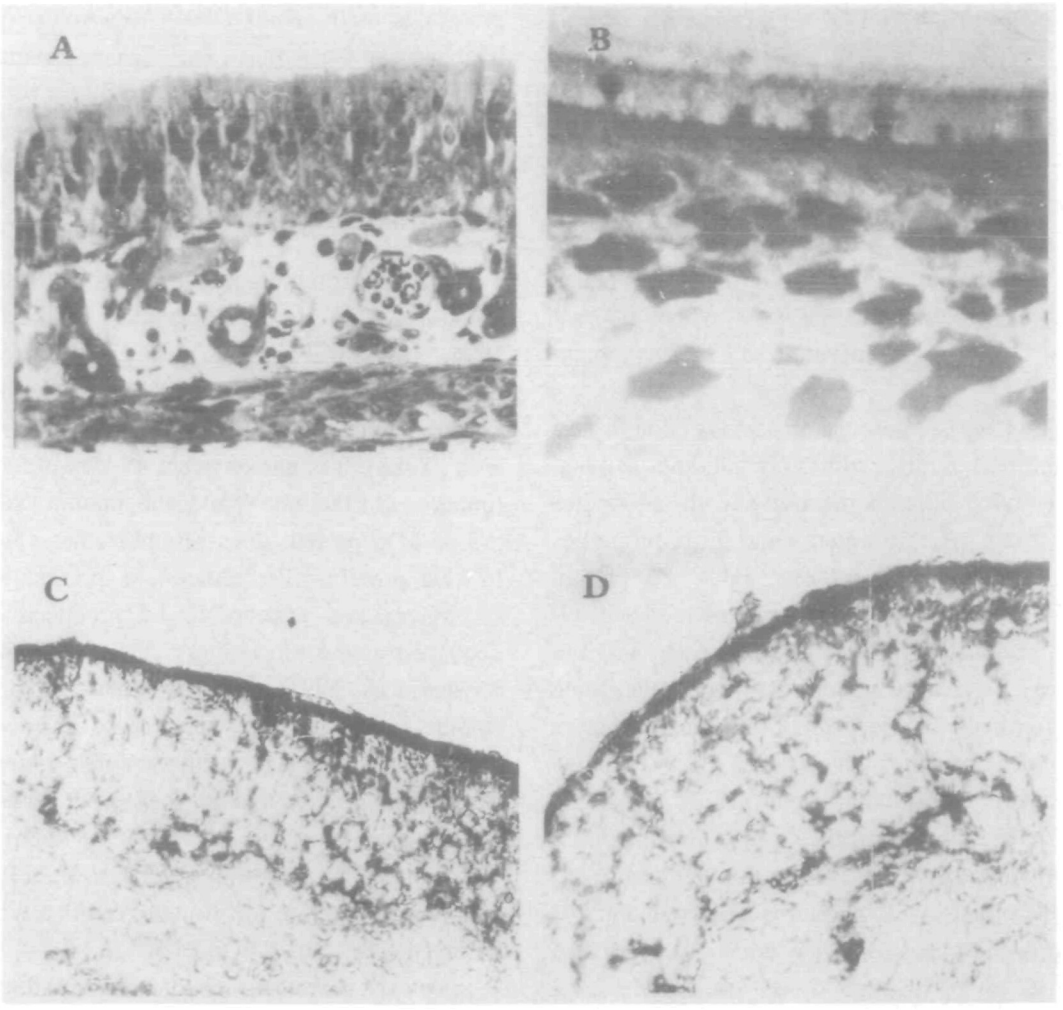

Figure 4 Immunohistochemical localization of 45-kDa protein. Antisera against 45-kDa protein. Coronal sections through the rat olfactory epithelium were stained with toluidine blue (A, B) and rabbit antiserum against 45-kDa protein (C, D). Antiserum reveals intense staining of the ciliary surface and apical part of the receptor and sustentacular cells

Photo-affinity labeling of 45-kDa protein by [^{32}P]GTP

Figure 5 shows the results of photo-affinity labelling of 45-kDa protein by [^{32}P]GTP. The 45-kDa protein has a high affinity to GTP, but not to ATP. It should be noted that in the water-soluble extract of rat olfactory mucosa, besides 45-kDa protein there is another high affinity GTP-binding protein with molecular mass about 55 kDa which we have not studied.

The purified 45-kDa protein also exhibits a low GTP hydrolytic activity. The apparent K_m for GTP in the basal GTPase reaction is 0.7–0.9 μM.

Thus, considering these properties of the isolated 45-kDa protein, we describe it as 'The water-soluble GTP-binding 45-kDa protein from olfactory epithelium'.

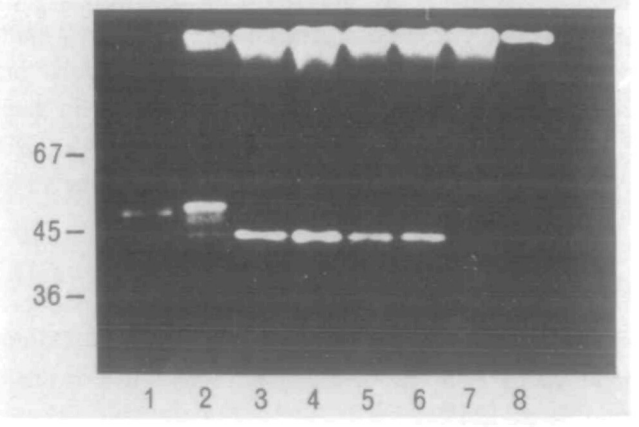

Figure 5 Photo-affinity labeling of 45 kDa protein by ^{32}P-GTP. (1) Triton X-100 extract of rat olfactory mucosa, (2) water-soluble extract of rat olfactory mucosa; (3) purified 45-kDa protein; (4) purified 45-kDa protein + 5 nm ATP; (5) purified 45-kDa protein + 5 μM ATP; (6) purified 45-kDa protein + 5 nM GTP; (7) purified 45-kDa protein + 5 μM GTP; (8) purified 45-kDa protein (without UV-radiation).

Discussion

We have identified a novel protein which appears to be located at the mucociliary surface. This is a water-soluble GTP-binding protein with a molecular mass of about 45-kDa (45-kDa protein). Its abundance in the mucosa (about 2% of total mucus protein) and the conspicuous localization of this protein near the sites where odorant recognition occurs, suggests an important role for this protein in olfaction.

At the beginning we supposed that 45-kDa protein is a member of G-protein family with GTP-binding activity, low GTP-ase activity, immunoreactivity with antibodies generated against α_{common}. However, some of its properties seem to disagree with this hypothesis. First, the protein staining of SDS-electrophoresis patterns of isolated G-proteins always revealed its subunit structure (α-, β- and γ-subunits) (Spiegel, 1988). In contrast, under the same conditions the purified 45-kDa protein was revealed as a single polypeptide and we have never seen putative β-, γ-subunits during our isolation procedure. It is unlikely that the 45-kDa protein dissociates completely from a hypothetical β/γ-complex. Secondly, the amount of the 45-kDa protein in the olfactory epithelium is very high and this protein is found in the mucus. So, it would be very strange for a G-protein to function outside a cell. Nevertheless, we have attempted to ribosilate the 45-kDa protein using cholera and pertussis toxins, but this was not successful. Finally, the antibodies against the purified 45-kDa did not react with the known G-proteins of the olfactory epithelium (Figure 3a,b).

Actin is one of the main proteins in the olfactory epithelium (Chen and Lancet, 1984) and has a molecular mass quite similar to that of our protein. In fact, actin cannot be distinguished from the 45-kDa protein under SDS-electrophoresis conditions, but it is unlikely that the 45-kDa protein is actin. Antibodies (Anti-Actin, A 2066, Sigma) which recognize actin in many species (ranging from amoeba to human) did not react with the 45-kDa protein. Moreover, immunohistochemical studies at the light microscopy level using antibodies against 45-kDa protein did not show that the 45-kDa protein is present in cell bodies of the olfactory epithelium.

The most prominent mucus protein with strong suggested function is a family of odor-binding proteins (OBP) (Pelosi, 1994). Their localization in nasal glands and mucus secretion of these glands suggests a role for OBP in concentrating and transporting odorants from the ambient air (Pevsner *et al.*, 1988). It is unlikely that 45-kDa protein has the same function. On the other hand, the amount and localization of the 45-kDa protein does resemble that of olfactomedin, a 57-kDa protein. Olfactomedin is present in the olfactory epithelium and appears to be produced by glands and deposited at the mucociliary surface (Anholt *et al.*, 1990; Snyder *et al.*, 1991). Our preliminary immunohistochemical studies at the electron microscopic level showed that the 45-kDa protein is also localized in the secretory granules of sustentacular cells and in the lower level of the mucus surrounding olfactory cilia, dendritic knobs, and microvilli of sustentacular cells (Novoselov *et al.*, 1995). So, the 45-kDa protein may be functionally coupled with olfactomedin during the early events of olfaction.

Recently, it was found that GTP-binding proteins took part in exocytosis of neurotransmitters in neurons and secretory cells (Balch, 1990; Rothman, 1992; Anhert-Hilger *et al.*, 1994). In this case, it is possible that the GTP-binding 45-kDa protein plays a part in the exocytosis of substances into the olfactory mucus.

So, the function of 45-kDa protein remains to be determined. Further investigations including the elucidation of its primary structure will eventually clarify the role of this novel protein in olfactory reception.

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REFERENCES

Ahnert-Hilger, G., Schafer, T., Spicher, K., Grund, C., Schultz, G. and Wiedenmann, B. (1994) Detection of G-protein heterotrimers on large dense core and small synaptic vesicles of neuroendocrine and neuronal cells. *Eur. J. Cell Biol.*, **65**, 26–38.

Anholt, R.R.H., Petro, A.E. and Rivers, A.M. (1990) Identification of a group of novel membrane proteins unique to chemosensory cilia of olfactory receptor cells. *Biochemistry*, **29,** 3366–3373.

Antonoff, R.S., Ferguson, J.J. and Idelkope, G. (1976) Direct photoaffinity labeling of cyclic nucleotide binding proteins with guanosine-3'5'monophosphate. *Photochem. Photobiol.*, **23,** 327–329.

Baehr, W., Morita, E.A., Swanson, R.J. and Applebury, M.L. (1982) Characterization of bovine rod outer segment G-protein. *J. Biol. Chem.*, **257,** 6452–6460.

Balch, W.E. (1990) Small GTP-binding proteins in vesicular transport. *Trends Biochem. Sci.*, **15,** 473–477.

Bignetti, E., Cavaggaioni, A., Pelosi, P., Persaud, K.C., Sorbi, R.T. and Tirindelli, R. (1985) Purification and characterization of an odorant-binding protein from cow nasal tissue. *Eur. J. Biochem.*, **149,** 227–231.

Bradford, M.M. (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye finding. *Anal. Biochem.*, **72,** 248–254.

Breer, H., Boekhoff, I. and Tareilus, E. (1990) Rapid kinetics of second messenger formation in olfactory transduction. *Nature*, **345,** 65–68.

Buck, L. and Axel, R. (1991) A novel multigene family may encode odorant receptor: a molecular basis for recognition. *Cell*, **65,** 175–187.

Chen, Z. and Lancet, D. (1984) Membrane proteins unique to vertebrate olfactory cilia: candidate for sensory receptor molecules. *Proc. Nat. Acad. Sci. USA*, **81,** 1859–1863.

Chen, Z., Pace, U., Rohen, D. and Lancet, D. (1986) Polypeptide gp95. A unique glycoprotein of olfactory cilia with transmembrane receptor properties. *J. Biol. Chem.*, **261,** 1299–1305.

Fesenko, E.E., Novoselov, V.I. and Bystrova, M.F. (1988) Properties of odor binding glycoproteins from rat olfactory epithelium. *Biochim. Biophys. Acta*, **937,** 369–378.

Fulton, A. (1984) *The Cytoskeleton. Cellular architecture and choreography.* Chapman and Hall Ltd, London.

Green, N., Alexander, H., Olson, A., Alexander, S., Shinnik, T.M., Sutcliffe, J.G. and Lerner, R.A. (1982) Immunogenic structure of the influenza virus hemagglutinin. *Cell*, **28,** 477–487.

Jones, D.T. and Reed, R.R. (1989) G_{olf}: an olfactory neuron specific G-protein involved in odorant signal transduction. *Science*, **24,** 790–795.

Jones, D.T., Masters, S.B., BOurne, H.R. and Reed, R.R. (1990) Biochemical characterization of three stimulatory GTP-binding proteins. The large and small forms of G_s and olfactory-specific G-protein, G_{olf}. *J. Biol. Chem.*, **265,** 2671–2676.

Krieger, J., Schleichner, S., Strotman, J., Wanner, I., Boekhoff, I., Raming, K., deGeus P. and Breer, H. (1994) Probing olfactory receptors with sequence-specific antibodies. *Eur. J. Biochem.*, **219,** 829–836.

Laemmli, U.K. (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature*, **227,** 680–685.

Mumby, S.M., Kahn, R.A., Manning, D.R. and Gilman, A.G. (1986) Antisera of designed specificity for subunits of guanine nucleotide-binding regulatory proteins. *Proc. Nat. Acad. Sci. USA*, **83,** 256–269.

Ngai, J., Dowling, M M., Buck, L., Axel, R. and Chess, A. (1993) The family of genes encoding odorant receptors in the channel catfish. *Cell*, **72,** 657–666.

Ngai, J., Chess, A., Dowling, M.M., Necles, N., Macagno, E.R. and Axel,R. (1993) Coding of olfactory information—topography of odorant receptor expression in the catfish. *Cell*, **72,** 667–680.

Novoselov, V.I., Krapivinskaya, L.D. and Fesenko, E.E. (1988) Amino acid binding proteins from the olfactory epithelium of skate (*Dasyatis pastinaca*). *Chem. Senses*, **13,** 267–278.

Novoselov, V.I., Krapivinskaja, L.D., Krapivinsky, G.B. and Fesenko E.E. (1989) GTF-binding proteins from skate olfactory epithelium associated with odor binding proteins. *FEBS Lett.*, **219,** 197–201.

Novoselov, V.I., Peshenko, I.V., Evdokimov, V.A., Nikolaev, J.V., Matveeva, E.A. and Fesenko, E.E. (1994) Water-soluble GTP-binding protein from rat olfactory epithelium. *FEBS Lett.*, **353,** 286–288.

Novoselov, V.I., Peshenko, I.V, Evdokimov, V.A., Nikolaev, J.V., Popov, V.I., Matveeva, E.A. and Fesenko, E.E. (1995) Some properties of the specific GTP-binding protein from rat olfactory epithelium. *Sensor System*, **9,** 85–95 (in Russian).

Pelosi, P. (1994) Odorant-binding proteins. *Crit. Rev. Biochem. Mol. Biol.*, **29,** 199–228.

Pevsner, J., Hwang, P.M., Sklar, P.B., Venable, J.C. and Snyder, S.H. (1988) Odorant-binding protein and its mRNA are localized to lateral nasal gland implying a carrier function. *Proc. Nat. Acad. Sci. USA*, **85,** 2383–2387.

Raming, K., Krieger, J., Strotmann, J., Boekholt, I., Kubick, S., Baumstark, C. and Breer, H. (1993) Cloning and expression of odorant receptors. *Nature*, **361**, 353–356.

Ressler, K.J., Sullivan, S.L. and Buck, L.B. (1993) A zonal organization of odorant receptor gene expression. *Cell*, **73,** 597–609.

Rothman, J.E. (1992) Molecular dissection of the secretory pathway. *Nature*, **355,** 409–415.

Snyder, D.A., Rivers, A.M., Yokoe, H., Menco, B.Ph.M. and Anholt, R.R.H. (1991) Olfactomedin: purification, characterization, and localization of a novel olfactory glycoprotein. *Biochemistry*, **30,** 9143–9153.

Spiegel, A.N. (1988) Guanine nucleotide binding proteins and signal transduction. *Vitamins and Hormones*, **44,** 44–101.

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