Membrane receptor for odour-binding proteins

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Specific binding of ^{125}I -labelled bovine odour-binding protein (OBP) to isolated membranes from nasal mucosa was demonstrated. The interaction reached equilibrium within 30 min at 37 °C and was reversible. A Scatchard analysis of the equilibrium binding revealed a single population of binding sites, with the calculated equilibrium dissociation constant and maximum number of binding sites being $2.25 \pm 0.5 \mu\text{M}$ and 18.5 ± 2 pmol/mg of membrane protein respectively ($n = 2$). Receptor activity was decreased on digestion by trypsin, proteinase K or endoglycosidase H, was heat labile and was sensitive

to thiol-group-specific reagents. With the exception of rat and mouse major urinary proteins, which exhibit a high degree of structural similarity with OBP and bind similar ligands, other members of the lipocalin family, such as retinol-binding protein and β -lactoglobulin, failed to inhibit the binding of ^{125}I -labelled OBP to its receptor. The receptor seems not to be restricted to olfactory tissues, as it was detected in a variety of other tissues. This suggests that OBP is unlikely to play a role only in olfactory signal transduction. It might have a much broader role within the body; possibilities include a role in detoxification or signalling.

INTRODUCTION

Early searches for proteins responsible for olfactory signal transduction led to the independent discovery and purification of odour-binding protein (OBP) from cow [1] and rat [2] nasal mucosa. Although OBP is capable of binding odorants specifically, its inability to discriminate between the different classes of odorants and its soluble nature soon led to the conclusion that OBP is unlikely to be a classical olfactory receptor. The recent identification by molecular cloning of a large family of G-protein-linked odorant receptors from olfactory neuroepithelium [3] seems to confirm this conclusion. The presence of OBP in large amounts exclusively in olfactory and respiratory mucosal tissues does, however, suggest at least a role in some kind of olfactory process. This is reinforced by more recent reports describing the occurrence of more than one form of OBP in olfactory mucosa [4], each perhaps with some degree of odorant specificity. Moreover, some species, particularly rodents, possess substantial amounts of a large family of closely related binding proteins [5].

Several possible roles have been suggested for OBP. First, it might participate in olfaction by facilitating the movement of hydrophobic odorants across the aqueous mucus layer to access the membrane-bound receptors of olfactory neurons [6]. Perhaps OBP might even make a specific interaction with the olfactory receptor, although this seems unlikely. Secondly, it might function in the termination of the olfactory signal by 'removing' odorants from the receptors once the receptors have been stimulated [6,7]. Thirdly, these proteins might serve a primary defence mechanism by recognizing and removing potentially harmful substances from the mucosa. Lastly, they may function as 'scavenger' molecules by assisting in the clearance of excess odorant from the mucus layer to enable new stimuli to be received [8].

OBP is synthesized in lateral nasal glands and secreted into the nasal mucosa. It is a homodimer with a subunit molecular mass of 18 kDa. A partial primary sequence revealed that the protein was very closely related to rodent urinary proteins of unknown

function [9,10]. The complete primary amino acid sequences of OBPs from various species were subsequently reported [4,11,12], which revealed that OBP belongs to a large superfamily of ligand-binding proteins that exhibit a range of different specificities. Members of this family called lipocalins are characterized by a common structural framework consisting of eight anti-parallel β -strands that fold to form a β -barrel. Inside this barrel lies the ligand binding site (reviewed in [13,14]). The residues lining the binding site differ between the members and define the specificity for the ligand. On the basis of this structural similarity, it has been suggested that OBP might play a role similar to retinol-binding protein (RBP) by facilitating the uptake of its ligand across the plasma membrane [15]. It is now well established (reviewed in [14,16]) that RBP transports the hydrophobic retinol molecule in the serum and other aqueous compartments and delivers it to cells via interaction with a specific membrane receptor. We report here specific receptors for OBP in olfactory and respiratory epithelial membranes and discuss their possible physiological role.

MATERIALS AND METHODS

Carrier-free Na^{125}I was obtained from Amersham International plc. Enzymobeads were purchased from Bio-Rad Laboratories. β -Lactoglobulin, human serum albumin, endoglycosidase H, neuraminidase, trypsin, proteinase K, *p*-chloromercuribenzenesulphonic acid (PCMBS), iodoacetate, dithiothreitol (DTT), 2-isobutyl-3-methoxypyrazine and dibutyl phthalate were obtained from Sigma Chemical Co. Dinonyl phthalate was from BDH. 2-Isobutyl-3-[^3H]methoxypyrazine (1.8 Ci/mmol) was a gift from Professor A. Cavaggioni (University of Parma, Parma, Italy). All other chemicals were obtained at the appropriate grade from Sigma or BDH Chemicals. Olfactory and respiratory mucosa were dissected from cow heads in the Bradford abattoir.

Preparation of membranes

Olfactory epithelial membranes and chemosensory cilia were prepared as described by Chen et al. [17]. Olfactory or respiratory

Abbreviations used: B_{max} , maximum number of binding sites; DTT, dithiothreitol; K_{d} , dissociation constant; MUP, mouse major urinary protein; OBP, odour-binding protein; PCMBS, *p*-chloromercuribenzenesulphonic acid; RBP, retinol-binding protein.

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epithelial tissue collected from two cow heads was minced with scissors in buffer A (30 mM Tris/HCl, pH 8.0, containing 150 mM NaCl, 2 mM EDTA and 1 mM PMSF) and homogenized with a Polytron homogenizer three times for 5 s each at maximum speed. Solid CaCl_2 was added to a final concentration of 10 mM, and the suspension was incubated at 4 °C with gentle stirring for 20 min. After this time the suspension was centrifuged at 1700 *g* for 5 min, the supernatant was collected and the cilia pelleted at 10000 *g* for 5 min. The supernatants were recentrifuged at 20000 *g* for 30 min. All centrifugation steps were performed at 4 °C. The final pellet consisted mainly of olfactory epithelial membranes. The protein concentration of the final preparations was estimated by the method of Markwell et al. [18]. The isolated membranes and cilia were stored at -70 °C as suitable aliquots at a concentration of 5 mg/ml protein in PBS (10 mM sodium phosphate, 150 mM NaCl, pH 7.4).

Purification of OBP

OBP was purified from the bovine olfactory and respiratory mucosal tissue as described by Tirindelli et al. [12]. At the end of this procedure the protein was approx. 98 % pure. Further purification was performed on a Mono-Q column (0.5 cm × 5 cm; Pharmacia) with a 0–1 M NaCl gradient in 50 mM Tris/HCl, pH 7.8. OBP was eluted at approx. 0.34 M NaCl concentration. The odorant binding activity of the purified protein was assayed as described by Bignetti et al. [1] with 2-isobutyl-3-[^3H]methoxypyrazine as the ligand. OBP (40 μM) was incubated with 2×10^4 d.p.m. of 2-isobutyl-3-[^3H]methoxypyrazine in 50 mM Tris/HCl/0.15 M NaCl, pH 7.8, for 5 min at 4 °C in a final volume of 250 μl . The mixture was applied to a 1.5 ml Eppendorf tube packed with 1 ml of Sephadex G-10 resin equilibrated with buffer A. The tube was centrifuged at 200 *g* at room temperature for 1–2 min with a Baird & Tatlock Mark IV refrigerated centrifuge. The flow-through was collected into a scintillation vial and counted for radioactivity with Packard 1900 TR liquid-scintillation analyser. The radioactivity measured represented the total binding of 2-isobutyl-3-[^3H]methoxypyrazine to OBP. Non-specific binding was estimated in parallel incubations in the presence of 100 μM unlabelled ligand (2-isobutyl-3-methoxypyrazine) and the background binding by replacing the OBP solution with buffer. Specific binding was calculated by subtracting non-specific from total binding.

Radioiodination of OBP

OBP was labelled with ^{125}I with Enzymobeads in accordance with the instructions of the manufacturer. ^{125}I -labelled OBP was separated from free ^{125}I by gel filtration on a Sephadex G-50 column (0.9 cm × 30 cm) equilibrated with PBS containing 0.2 % ovalbumin. The specific radioactivity of the labelled OBP ranged from 190 to 300 Ci/mmol, with more than 98 % of the radioactivity being precipitable by trichloroacetic acid. ^{125}I -labelled OBP was stored at -20 °C as aliquots.

Binding of ^{125}I -labelled OBP to olfactory mucosal membranes

Binding of ^{125}I -labelled OBP to olfactory mucosal membranes was performed with the oil centrifugation assay described by Sivaprasadarao and Findlay [19] with a minor modification. Frozen membranes or cilia were thawed by incubating at 37 °C for 5 min and then washed twice at 4 °C with 20 vol. of assay buffer (0.2 % ovalbumin in PBS). This would remove any residual OBP that might have been left with the membranes during purification. Washed membranes (200–250 μg) were incubated with ^{125}I -labelled OBP (1–2 nM) in assay buffer in a total volume

of 100 μl . After incubation for 1 h at 37 °C the tubes were centrifuged for 2 min at 12500 *g* at room temperature and then were overlaid with a 3:2 (v/v) mixture of dibutyl phthalate and dinonyl phthalate and centrifuged for a further 2 min at 12500 *g* at room temperature. The tubes were frozen in a solid CO_2 /ethanol bath and the bottoms of the tubes containing the pellet were subsequently cut off and counted for radioactivity. This gave the total binding. Parallel incubations in the presence of excess (10 μM) unlabelled OBP were performed to determine non-specific binding. Specific binding was calculated by subtracting non-specific from total binding. All the assays were performed in triplicate and at least twice. The standard deviation of triplicate assays is normally within 10 %.

RESULTS

Binding of ^{125}I -labelled OBP to epithelial membranes and cilia

Purified OBP was homogeneous with an apparent molecular mass of 19 kDa on SDS/PAGE (results not shown) and showed specific odorant binding activity with 2-butyl-3-[^3H]methoxypyrazine (Table 1). To identify receptors for OBP the purified protein was labelled with ^{125}I and incubated with membranes and cilia prepared from bovine olfactory and respiratory epithelia. The membrane-bound ^{125}I -labelled OBP was then separated from the unbound label by oil centrifugation [19]. Table 2 shows that the binding to olfactory and respiratory membranes was decreased by 30–40 % when excess unlabelled OBP was included in the reaction, indicating that the labelled and the unlabelled OBP competed for the same specific binding sites on these membranes. Specific binding to olfactory cilia, however, was found to be insignificant.

Table 1 Binding of 2-isobutyl-3-[^3H]methoxypyrazine to OBP

The assay of binding of odorant to OBP was performed in duplicate as described in the Materials and methods section. Means $\pm$ S.D. of data from triplicate analyses are given for one set of experiments. The experiment was done twice.

	Binding (d.p.m.)
Total	3593 $\pm$ 113
Non-specific	1667 $\pm$ 123
Background	1427 $\pm$ 65
Specific	1926 $\pm$ 167

Table 2 Assay of ^{125}I -labelled OBP binding to membranes from bovine nasal cavity tissues

Respiratory and olfactory epithelial membranes (5 mg/ml each) and olfactory cilia (2 mg/ml) were assayed for binding of ^{125}I -labelled OBP (2.2 nM) at 37 °C in the absence (total binding) or presence (non-specific binding) of 10 μM unlabelled OBP. The binding assay was terminated as described in the Materials and methods section. Means $\pm$ S.D. of data from triplicate analyses are given for one set of experiments. The experiment was done three times.

Membrane	Binding (c.p.m.)		
	Total	Non-specific	Specific
Respiratory epithelia	4354 $\pm$ 171	2947 $\pm$ 311	1407 $\pm$ 354
Olfactory epithelia	4367 $\pm$ 26	3255 $\pm$ 104	1112 $\pm$ 107
Olfactory cilia	3467 $\pm$ 863	3256 $\pm$ 204	211 $\pm$ 886

Figure 1 Time and temperature dependence of ^{125}I -labelled OBP binding to respiratory epithelial membranes

Respiratory epithelial membranes (2.5 mg/ml protein) were incubated with ^{125}I -labelled OBP (8 nM) in the presence or absence of $10\text{ }\mu\text{M}$ unlabelled OBP at $4\text{ }^{\circ}\text{C}$ (■), $22\text{ }^{\circ}\text{C}$ (●) or $37\text{ }^{\circ}\text{C}$ (▲) in a final volume of 2.5 ml. At the time points indicated, $100\text{ }\mu\text{l}$ aliquots (triplicate) were removed and binding was determined as described in the Materials and methods section. Means $\pm$ S.D. of data from triplicate analyses are given for one set of experiments. The experiment was done twice.

Figure 2 Dissociation of ^{125}I -labelled OBP from respiratory epithelial membranes

Respiratory epithelial membranes (1 mg/ml) were incubated for 1 h with ^{125}I -labelled OBP (2.1 nM). Dissociation was then induced by the addition of unlabelled OBP to $10\text{ }\mu\text{M}$ and terminated by oil centrifugation at the times indicated. The remaining ^{125}I -labelled OBP bound to the membrane was plotted as a function of time. Means $\pm$ S.D. of data from triplicate analyses are given for one set of experiments. The experiment was done twice. Inset: data points between 0 and 15 min were plotted as $\ln(B_t/B_{eq})$ on the ordinate and time t on the abscissa (inset), where B_t is the binding remaining at time t , and B_{eq} is the equilibrium binding. The slope is $0.003 \pm 0.00015/\text{min}$ and the intercept $-0.047 \pm 0.002/\text{min}$. The standard error of the fit is 0.0985.

Time, temperature and pH dependence of ^{125}I -labelled OBP binding

Characterization of the OBP binding sites was performed with respiratory epithelial membranes. The membranes were incubated with ^{125}I -labelled OBP at 4 , 22 and $37\text{ }^{\circ}\text{C}$ for various lengths of time and the specific binding at each time point was determined. Binding at 22 and $37\text{ }^{\circ}\text{C}$ reached equilibrium within 30 min of incubation and remained stable thereafter (Figure 1). No difference in the equilibrium binding values at these two temperatures was noticed, although binding reached equilibrium

Figure 3 Competitive inhibition of ^{125}I -labelled OBP binding to respiratory epithelial membranes

Respiratory epithelial membranes (2.5 mg/ml protein) were incubated at $37\text{ }^{\circ}\text{C}$ for 3 h with 2.1 nM ^{125}I -labelled OBP and various concentrations of unlabelled OBP. The binding assay was terminated as described in the Materials and methods section. Data from the competition curve were converted into Scatchard form (inset) and fitted by linear regression analysis at the 95% confidence limit. The calculated K_d and B_{max} values are $2.25 \pm 0.5\text{ }\mu\text{M}$ and $18.5 \pm 2\text{ pmol/mg}$ of membrane protein respectively. Results shown are derived from one experiment. The experiment was done twice.

more rapidly at $37\text{ }^{\circ}\text{C}$ than at $22\text{ }^{\circ}\text{C}$. Binding at $4\text{ }^{\circ}\text{C}$, in contrast, reached equilibrium very rapidly (approx. 20 min). However, the equilibrium binding at this temperature represented only 33% of that at 22 and $37\text{ }^{\circ}\text{C}$. Non-specific binding showed no time or temperature dependence (results not shown). Maximal binding of ^{125}I -labelled OBP to the membranes occurred at pH 7.0 and at physiological concentrations of NaCl. The binding was decreased by over 60% at pH 10.0 and by about 20% at pH 5.0. In the presence of 10 mM sodium phosphate (pH 7.4) alone, specific binding was decreased by over 50%.

Dissociation of ^{125}I -labelled OBP binding

Respiratory epithelial membranes were first incubated with ^{125}I -labelled OBP for 1 h at $37\text{ }^{\circ}\text{C}$ to allow binding to reach equilibrium. Dissociation was then induced by the addition of a 5000-fold molar excess of unlabelled OBP. The dissociation curve (Figure 2) was monophasic, suggesting that only one membrane component was involved in specific binding. The dissociation rate constant was calculated to be $3 \times 10^{-3}/\text{min}$, which corresponds to a half-time of dissociation of 23 min.

Competitive inhibition of ^{125}I -labelled OBP binding by unlabelled OBP

Figure 3 shows that the addition of increasing concentrations of native OBP resulted in a progressive decrease in the equilibrium binding of ^{125}I -labelled OBP to respiratory epithelial membranes. When the competition data were transformed and plotted in Scatchard form [19], the resultant plot (Figure 3, inset) was linear, suggesting a single class of binding site. The calculated equilibrium dissociation constant (K_d) and the maximum number of binding sites (B_{max}) were $2.25 \pm 0.5\text{ }\mu\text{M}$ and $18.5 \pm 2\text{ pmol/mg}$ of membrane protein respectively ($n = 2$).

Effect of various treatments on ^{125}I -labelled OBP binding

Binding of OBP to membranes increased with the increase in membrane protein concentration, suggesting that the binding is

Figure 4 Effect of trypsin and proteinase K digestions on ^{125}I -labelled OBP binding activity of respiratory epithelial membranes

Respiratory epithelial membranes (5 mg/ml) were incubated at 37 °C with 0.2 mg/ml trypsin (●) or 0.2 mg/ml proteinase K (□). Aliquots of membranes were removed at the times indicated and the enzymic digestion was terminated by adding PMSF to a final concentration of 0.1 mM. As a control (○), membranes were incubated with proteinase K in the presence of 1 mM PMSF and the binding activity was determined at the times indicated by using 2.2 nM ^{125}I -labelled OBP. Means $\pm$ S.D. of data from triplicate analyses are given for one set of experiments. The experiment was done twice.

Table 3 Effect of various treatments on ^{125}I -labelled OBP binding to respiratory epithelial membranes

Respiratory epithelial membranes (5 mg/ml) were washed with 50 mM sodium acetate buffer, pH 5.0, and then incubated at 37 °C for 3 h in the presence or absence of endoglycosidase H (4.5 m-units/ml) or neuraminidase (1 m-unit/ml). To examine the effects of protein modification agents, membranes were incubated with different modifying reagents at the indicated concentrations for 10 min at 37 °C. These reagents were added either individually or in combination as indicated. The treated membranes were washed three times with binding assay buffer and then assayed for binding activity, as described in the Materials and methods section, with 2.1 nM ^{125}I -labelled OBP. In controls, membranes were treated with the corresponding buffer but free from any additives. For thermal inactivation studies, membranes (5 mg/ml protein) were heated at 80 °C for 10 min and then cooled on ice before the binding assay. Total binding was 4554 ± 162 . Means $\pm$ S.D. of data from triplicate analyses are given for one set of experiments. The experiment was done twice.

Reagent	Concentration (mM)	Specific binding (c.p.m.)	Percentage binding
Control		1557 ± 238	100
Endoglycosidase H		1026 ± 141	65
Neuraminidase		1712 ± 150	110
DTT	2	1575 ± 212	101
PCMBs	2	795 ± 244	51
DTT + PCMBs	2, 2	1238 ± 258	79
EDTA	10	1588 ± 361	102
Iodoacetic acid	5	1136 ± 263	73
Heat at 80 °C		848 ± 82	54

protein associated. When epithelial membranes were treated with trypsin (0.2 mg/ml) or proteinase K (0.2 mg/ml) at 37 °C, 15% and 45% respectively of OBP binding was destroyed within 1 h (Figure 4). Heating the membranes at 80 °C for 10 min decreased ^{125}I -labelled OBP binding activity by 46% (Table 3). These results confirm that the OBP binding site is on a membrane protein. Treatment of membranes with endoglycosidase H also decreased the binding activity (Table 3), indicating that the OBP binding protein might include a glycosylated component.

Table 4 Ligand specificity of ^{125}I -labelled OBP binding to respiratory epithelial membranes

Respiratory epithelial membranes (4.5 mg/ml) were incubated with ^{125}I -labelled OBP (2.3 nM) at 37 °C for 1 h in the presence or absence of competing proteins at the concentrations indicated. The inhibition obtained with 10 μM OBP was taken as 100% and the rest of the values are expressed with reference to this. Means $\pm$ S.D. of data from triplicate analyses are given for one set of experiments. The experiment was done twice.

Protein	Concentration (μM)	Total binding (c.p.m.)	Percentage inhibition
None	—	5597 ± 230	0
OBP	10	3801 ± 143	100
MUP	10	3480 ± 152	117
RBP	5	5462 ± 81	7.5
β -Lactoglobulin	10	5567 ± 169	1.6
Human serum albumin	10	5447 ± 255	8.3

Table 3 also shows the effect of protein-modifying reagents on the binding activity. EDTA had no effect, indicating that external bivalent cations such as Ca^{2+} or Mg^{2+} are probably not required. Similarly, treatment of membranes with DTT had no effect on the binding, suggesting that disulphide bridges are either absent from the receptor or are not crucial to the binding activity of the receptor. Treatment of the membranes with PCMBs, however, inhibited the binding by 50%. This effect was abolished in the presence of DTT. This, together with the fact that iodoacetic acid treatment of the membrane caused some inhibition of binding, indicates the possible presence of one or more sensitive thiol residues on the receptor.

Specificity of ^{125}I -labelled OBP binding

To assess the specificity of the binding, competition experiments were performed with various unlabelled proteins (Table 4). Except for OBP itself and the closely related mouse major urinary protein (MUP), none of the other proteins tested was able to compete with ^{125}I -labelled OBP for binding to its receptor. When examined for tissue specificity, specific binding of ^{125}I -labelled OBP was also observed with membranes isolated from human placental brush border and several cell lines including HeLa cells, COS cells and skin fibroblasts (results not shown).

DISCUSSION

The amino acid sequence similarity and the overall three-dimensional structural similarity between OBP and RBP and other members of the lipocalin superfamily led to the proposition that OBP might have a biological role similar to that of RBP [12,15]. Accordingly, it was suggested that OBP might bind to specific membrane-bound receptors on target tissues and thereby deliver the odorant molecule that it carries.

In this study we have demonstrated the presence of specific receptors for OBP in olfactory and respiratory epithelial membranes. The binding of ^{125}I -labelled OBP to the membranes was dependent on time, temperature (Figure 1) and pH, and was reversible (Figure 2). Binding reached equilibrium rapidly at 37 °C. Contrary to the expected slow increase in binding, the binding at 4 °C also reached equilibrium rapidly, but the equilibrium binding at 4 °C represented only one-third of that observed at 37 °C. This could be due to the recruitment of new binding sites at 37 °C. Similar observations have been made with RBP receptor [19,20]. Increasing the concentration of unlabelled OBP inhibited the binding of the labelled OBP proportionately

(Figure 3). The value of K_d for the interaction of OBP with its receptor is $2.07 \pm 0.175 \mu\text{M}$ (Figure 3, inset). Although this value seems somewhat high, it is consistent with the high abundance of OBP in the nasal mucosa (consisting of at least 1% of soluble nasal protein) [1]. Whether the binding of odour molecule changes the affinity of OBP for its receptor is not clear at present.

The ^{125}I -labelled OBP binding activity of the membranes is sensitive to trypsin, proteinase K, endoglycosidase H, heat and protein modification reagents including PCMBs and iodoacetic acid, suggesting that a glycoprotein is responsible. The binding site seems to be very specific. No other member of the lipocalin superfamily tested, with the exception of MUP, could substitute for OBP in competing for the binding site. The urinary protein exhibits a high degree of structural [21] as well as functional [9] similarity to OBP. Both proteins bind a wide range of odorants, although their sites of synthesis and tissue distribution are different. MUP is synthesized in the liver and secreted into the plasma. In male rats and mice these proteins bind pheromones and are secreted in the urine. There is evidence [22] to suggest that they influence behavioural and sexual responses by reaching the olfactory-related vomero-nasal organ of their female counterparts after licking.

The role of OBP in olfaction is not clear. It was suggested that OBP might play a role in olfaction by presenting the odour molecules to odorant receptors of sensory cilia or in the termination of olfactory signals by removing the odorants from the receptor once the receptor has been stimulated [6,7]. However, the apparent absence of the OBP receptor in significant amounts in olfactory cilia and its presence in large amounts in respiratory membranes (Table 2) suggests that OBP probably makes no direct contact with the odorant receptors. Others [8,23] have suggested that it is involved in the detoxification of excess odorant. Accordingly, OBP may bind to its receptor to facilitate the transfer of the odour molecules to the endoplasmic reticulum of the cell, where they undergo detoxification. Our observation that receptors for OBP are present in a number of tissues unrelated to olfaction suggests that OBP plays a much more general role. Thus OBP- or MUP-related proteins might be involved (via OBP receptors) in the general defence mechanism of the body by removing potentially toxic substances that could find entry via nasal, respiratory and intestinal pathways, and thence into the bloodstream. These binding proteins might be synthesized in tissues such as liver and secreted into the bloodstream, where they pick up their ligand and then present them to the intracellular detoxification machinery. The existence of a high concentration of OBP in the nasal and respiratory tissues might be explained by the fact that these are the first sites to encounter odour and other potentially toxic substances from the environment.

The only other member of the lipocalin superfamily for which detailed information on the cell-surface receptor is available is plasma RBP. There is now considerable evidence (reviewed in [14,16]) to suggest that RBP binds to the receptor with high affinity (K_d approx. 50 nM) and releases its bound retinol to the cell. The resultant apo-RBP is believed to lose its affinity for the receptor and to dissociate quickly from the receptor, thereby

vacating the site for a second RBP molecule to bind. The existence of specific receptors for OBP suggests that a similar mechanism might operate if the bound odorant were to cross the cell membrane. Uptake experiments, employing OBP loaded with radioactive odorants, on intact cells or membrane vesicles is one way to test this possibility. For RBP it has been shown [24] that RBP binds to its receptor via the loops surrounding the entrance to its 'retinol binding pocket'. Unlike RBP, both OBP and MUP are homodimers. The crystallographic data [21] show that the loops surrounding the entrance to the ligand binding pocket are not involved in subunit-subunit interactions; instead they are exposed to the surface. Thus the loops in OBP seem to be ideally placed for interaction with the receptor to occur and to facilitate the transport of the bound ligand to the target cell. However, endocytosis of an OBP-odorant complex is an alternative possibility.

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