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Molecular Cloning of Putative Odorant-Binding and Odorant-Metabolizing Proteins†,‡

T. Neil Dear, Kathryn Campbell, and Terence H. Rabbitts*
MRC Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH, United Kingdom
Received July 8, 1991; Revised Manuscript Received August 15, 1991

ABSTRACT: Olfactory reception occurs via the interaction of odorants with the chemosensory cilia of the olfactory receptor cells located in the nasal epithelium. The cDNA clones from mRNA specific to olfactory mucosa were studied. One of these clones, OBP_{II}, encodes a secretory protein with significant homology to odorant-binding protein (OBP), a protein with broad odorant-binding ability, and is expressed in the lateral nasal gland, which is the site of expression of OBP. The OBP_{II} sequence also shows significant homology to the VEG protein, which is thought to be involved in taste transduction. OBP_{II} is a new member of the lipophilic molecule carrier protein family. The second cDNA clone encodes a novel homologue of glutathione peroxidase, an enzyme involved in cellular biotransformation pathways. Its expression appears to be localized to the Bowman's glands, the site of several previously identified olfactory-specific biotransformation enzymes.

The vertebrate olfactory system is capable of detecting and distinguishing a wide variety of foreign molecules (Anholt, 1987). Olfactory transduction is initiated when odorants enter the nasal cavity, where they interact with the cilia protruding from the dendritic tips of the chemosensory neurons. Interaction between odorants and putative ciliary receptors enhances adenylyl cyclase activity in the receptor neurons, resulting in increased levels of cAMP (Breer et al., 1990) or IP_3 (Boekhoff et al., 1990). Such increases in cAMP have been demonstrated to activate olfactory receptor neuron ion channels (Nakamura & Gold, 1987). Differential activation of these neurons by different odorants leads to distinct patterns of activity which are relayed to the olfactory lobes of the brain (Lancet, 1986).

The olfactory system is highly specialized, with olfactory-specific forms of adenylyl cyclase (Bakalyar & Reed, 1990), G_α (Jones & Reed, 1989), a cAMP-gated ion channel (Dhallan et al., 1990), and several putative detoxification enzymes (Nef et al., 1989; Lazard et al., 1991) having been identified. Recently, a novel multigene family of G-protein-coupled receptors has been identified (Buck & Axel, 1991). This family is highly diverse and restricted in expression to the OM,[1] suggesting that the members encode the ciliary receptor molecules. Initial attempts to identify receptor candidates led to the identification and purification of OBP (Bignetti et al., 1985; Pevsner et al., 1985), synthesized in the nasal glands (Lee et al., 1987; Pevsner et al., 1986, 1988a,b) and transported to the mucus layer bathing the neuroepithelium where it binds odorants (Pevsner et al., 1988a). The lack of alternative OBPs and its broad spectrum of odorant binding ability suggested that it played a nondiscriminatory role in olfaction, probably assisting hydrophobic odorants to traverse the hydrophilic mucus layer in order to access the ciliary receptors (Carr et al., 1990). However, the recent report of diversity in homologues of Lepidoptera pheromone-binding proteins (Vogt et al., 1990) intimates that such proteins may selectively bind odorants. In order to further characterize the olfactory process, we have used the technique of subtractive hybridization to identify OM-specific mRNAs (Dear et al., 1991). We now report two olfactory mucosa specific cDNAs which may be involved in odorant recognition and metabolism before and after olfactory receptor binding. One of these clones encodes a homologue of rat OBP, termed OBP_{II}, which like OBP is nasal gland specific. A second cDNA clone encodes a homologue of rat glutathione peroxidase, an enzyme involved in cellular detoxification pathways (Flohe, 1982). Its olfactory-specific nature and similarity to a detoxification enzyme suggest that it may contribute to the degradation of odorants subsequent to olfactory signal transduction.

Materials and Methods

RNA Preparation. Ten-week-old Fischer rats were sacrificed and the OM, which included the lateral nasal gland, was

†T.N.D. is the recipient of a University of Sydney Medical Foundation Travelling Research Fellowship.
‡The nucleotide sequences reported in this paper have been submitted to GenBank under Accession Numbers M76733 (RY2D1) and M76734 (RY2G12).
* To whom correspondence should be addressed.

[1] Abbreviations: OBP, odorant-binding protein; OM, olfactory mucosa; PCR, polymerase chain reaction; GSHPx, glutathione peroxidase.

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removed using forceps. Tissue samples were stored in liquid N_2 until required. Total RNA was extracted as described (Cathala et al., 1983).

cDNA Subtractive Hybridization and Library Construction. cDNA preparation and subtraction were performed as described (Dear et al., 1991). A total cDNA library from OM poly(A)$^+$ RNA was constructed in λgt10 using a cDNA cloning kit (Pharmacia, Uppsala, Sweden) according to the manufacturer's instructions.

Blot Hybridization Analysis. Northern and Southern blot procedures have been described (Boehm et al., 1988). The OBP DNA probe corresponds to nucleotides 92–485 in the published cDNA sequence (Pevsner et al., 1988b) and was prepared by PCR amplification (Saiki et al., 1988) from OM cDNA, followed by cloning into pBluescript.

Anchored PCR. Five micrograms of total RNA was reverse-transcribed into cDNA in a 20-μL reaction volume containing 50 mM Tris-HCl, pH 8.3, 150 mM KCl, 10 mM $MgCl_2$, 5 mM DTT, 0.5 mM dNTPs, 25 μg/mL oligo(dT), and 1000 units/mL AMV reverse transcriptase. After incubation for 45 min at 42 °C, the reaction was adjusted to 100 μL and stored at −20 °C. Amplification was performed with 1 μL of this cDNA in a 20-μL volume using *Taq* DNA polymerase (Promega) under conditions recommended by the manufacturer. Reaction conditions were 94 °C for 2 min, 60 °C for 1.5 min, and 72 °C for 2 min for 30 cycles. Subsequent manipulations were performed as described (Frohman et al., 1988) with minor modifications. Oligo(dT)-primed cDNA was G-tailed and subjected to successive rounds of amplification using nested backward primers for the sequence of interest and the forward primer 5′-GGAATTCTCGAGC_{17}-3′.

Sequence Analysis. cDNA clones were subcloned into pBluescript and DNA sequence was determined for both strands using the dideoxy chain-termination method with random sonicated clones (Bankier et al., 1987) and in conjunction with sequence-specific oligonucleotides. Protein and nucleic acid alignments were made using the FASTA algorithm (Pearson & Lipman, 1988). Complete cDNA sequences were assembled using the Staden sequence assembly program (Staden, 1990).

In Situ Hybridization. Six-day-old rat OM was removed and embedded in Tissue Tek (Miles, Elkhart, IN), and 20-μm sections were cut. The procedures for fixation, probe preparation, and hybridization are detailed elsewhere (Boehm et al., 1991).

Results

Isolation of OM-Specific Clones RY2G12 and RY2D1 and Tissue Distribution of mRNA. A cDNA subtraction library was prepared from sequences specific to rat olfactory mucosa by hybridizing rat olfactory mucosa cDNA against a 100-fold excess of Rat-2 fibroblast poly(A)$^+$ RNA. The subtracted cDNA was purified by hydroxylapatite chromatography and cloned into the vector λgt10. Differential screening of this library with ^{32}P-labeled cDNA from crude rat olfactory mucosa and Rat-2 fibroblasts revealed distinct groups of cDNA clones that were olfactory mucosa specific in their RNA expression patterns. A detailed description of these clones is provided elsewhere (Dear et al., 1991). Two of these clones, RY2G12 and RY2D1, identified mRNA species of 0.9 and 1.3 kb, respectively. In the tissues studied, expression of RY2G12 and RY2D1 was found to be restricted to olfactory mucosa with no detectable mRNA present in rat thymus, lung, brain, spleen, kidney, heart, and liver (Figure 1).

This RNA analysis is rather crude because tissue RNA is a heterogeneous mixture derived from diverse cell types. A

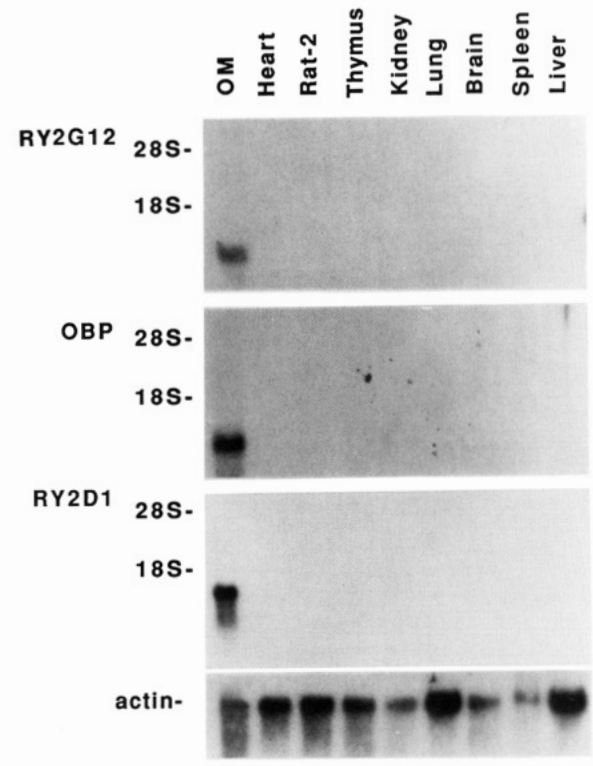

FIGURE 1: Expression of RY2G12 and RY2D1 mRNA. Northern hybridization analysis of RNA from different tissues and cell lines sequentially hybridized with RY2G12, OBP, RY2D1, and actin cDNA probes. Origin of RNA samples is indicated above each lane. OM = olfactory mucosal total RNA. The positions of the 28S and 18S rRNA markers are indicated.

precise sublocalization of mRNA within the olfactory tissue was achieved using in situ RNA hybridization (Figure 2). Anatomical regions of the mucosa were established first by thionine staining (Figure 2A) and second by in situ hybridization of cDNA probes corresponding to olfactory marker protein (OMP) (Figure 2B) and OBP (Figure 2D). The former is known to be specifically expressed in neurons within the sensory neuroepithelium (Danciger et al., 1989) establishing the location and limit of these cells within the sections used in this study. OBP, on the other hand, is synthesized in the lateral nasal (Steno's) gland (Pevsner et al., 1988a), which can be seen at the base of the septum (arrowed in Figure 2D). Hybridization of the cDNA clone RY2G12 (Figure 2C) parallels very closely that of the OBP since the majority of RY2G12 mRNA is localized within the lateral nasal gland. This colocalization with the site of OBP synthesis suggests common or complementary functions. The hybridization of the RY2D1 cDNA is quite distinct from any of the other probes, being localized in the subepithelial layer (Figure 2E,F). This layer contains the Bowman's secretory glands and the punctate pattern of the hybridization strongly suggests that the Bowman's glands are in fact the site of synthesis of RY2D1 mRNA.

Nucleotide Sequence of OM-Specific cDNAs. Full-length cDNAs for the clones RY2G12 and RY2D1 were obtained by screening a complete cDNA library made from unfractionated Fischer rat olfactory mucosa mRNA in conjunction with anchored PCR to obtain the complete 5′ sequence information. The sequences are shown in Figure 3 along with the predicted proteins encoded by these clones. The RY2G12 cDNA is 732 nucleotides in length and possesses an open reading frame of 176 amino acids encoding a putative protein with M_r 20 100. The initiating methionine of the RY2G12 predicted protein is followed by a stretch of hydrophobic amino

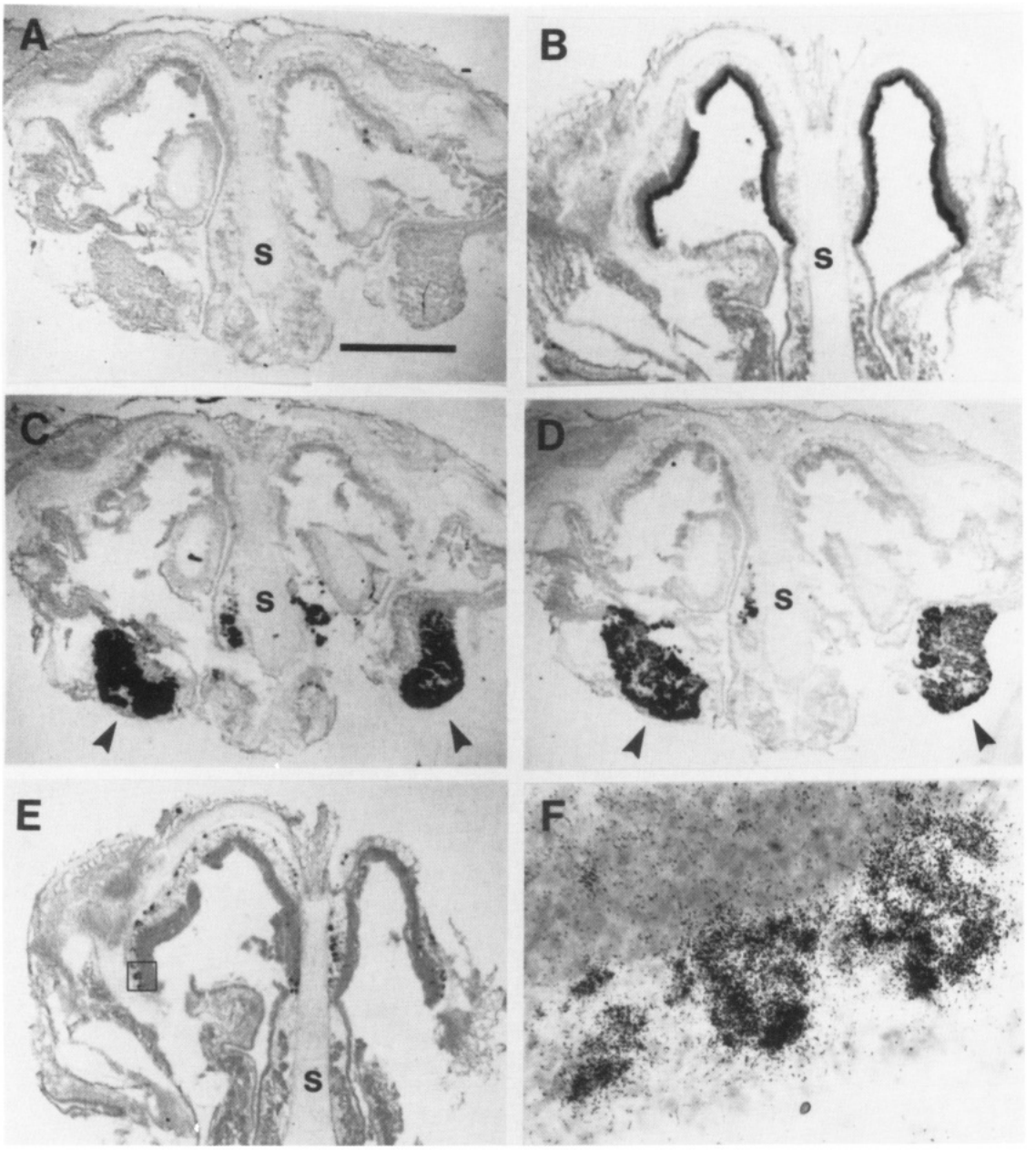

FIGURE 2: Tissue localization of RY2G12 and RY2D1 mRNA. Cross sections through Fischer rat olfactory epithelium were hybridized to ^{35}S-labeled cDNA probes followed by exposure to autoradiographic emulsion, after which the sections were counterstained with thionine. The septum (s) is indicated. Hybridization with ^{35}S-labeled sense cDNA did not reveal any specific signal (data not shown). Bar represents 1 mm. (A) An unhybridized thionine-stained section. (B) Hybridization of OMP; note specific hybridization signals in the neuroepithelium surrounding the nasal septum. (C) Hybridization of RY2G12. (D) Hybridization of OBP. (E,F) Hybridization of RY2D1. Panel E shows the punctate nature of hybridization in a layer beneath the neuroepithelium which is believed to be the Bowman's glands. Panel F is a 20× magnification of the boxed region in panel E; note that the top of photograph F is the position of the nasal lumen.

acids characteristic of secreted proteins, suggesting that this protein functions as a secretory molecule outside of the cell. The second cDNA, RY2D1, is 1151 nucleotides in length and possesses an open reading frame of 221 amino acids encoding a predicted protein of M_r 23 300.

Examination of the derived protein sequence of RY2G12 revealed homology to a group of soluble proteins that belong to a superfamily of lipophilic molecule carriers (Godovac-Zimmerman, 1988). Members of this family are involved in the binding and transport of small hydrophobic molecules including retinol, cholesterol, and various steroids (Schofield, 1988). Significant sequence identity was found to rat OBP (Pevsner et al., 1988b) (22.4% in 174 amino acids) and to a frog homologue of this protein termed BG (Lee et al., 1987) (18.3% in 169 amino acids). Maximal sequence homology was to rat VEG protein (41.8% in a 177 amino acid overlap), expressed in the Von Ebner's gland and believed to contribute to gustatory transduction by transporting sapid molecules to taste receptors (Schmale et al., 1990). Other homologies were to human α_1-microglobulin (Kaumeyer et al., 1986) (24.8% in 153 amino acids) and to rat retinol-binding protein (Laurent et al., 1985) (14.4% in 201 amino acids). Alignment of some of these sequences is shown in Figure 4a. The RY2G12 protein shares many of the conserved residues of this family of proteins including two cysteines at positions 79 and 171, which may participate in disulfide bonding, and the motif G-X-W-(X_n)-Y at positions 32-35 (Godovac-Zimmerman, 1988). Similarity between the RY2G12 and VEG genes was further emphasized by comparison of the nucleic acid sequences. Within the predicted protein coding regions of the

RY2G12

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                                        *       M K S R L L T V L L L G L M  14
ACACACTTCCAGGGTGAGCTGCCTTGTGTGAGAGCCCAGTGACTGGAGATGAAGAGCCGGCTCCTCACCGTCCTGCTGCTGGGGCTGATG  90

A V L K A Q E A P P D D Q E D F S G K W Y T K A T V C D R N  44
GCTGTCCTGAAGGCTCAGGAAGCCCCACCTGATGACCAGGAGGATTTCTCTGGGAAGTGGTACACAAAGGCCACGGTTTGTGACAGGAAC 180

H T D G K R P M K V F P M T V T A L E G G D L E V R I T F R  74
CACACAGATGGGAAGAGACCTATGAAAGTGTTCCCTATGACTGTGACAGCCCTGGAAGGAGGGGACTTAGAGGTCCGGATAACATTCCGG 270

G K G H C H L R R I T M H K T D E P G K Y T T F K G K K T F  104
GGGAAGGGTCATTGTCATTTGAGACGAATTACGATGCACAAAACTGATGAGCCTGGCAAGTACACTACCTTCAAAGGCAAGAAGACCTTC 360

Y T K E I P V K D H Y I F Y I K G Q R H G K S Y L K G K L V  134
TATACTAAGGAGATTCCTGTAAAGGACCACTACATCTTCTACATTAAAGGCCAGCGCCATGGGAAATCATATCTGAAGGGGAAACTCGTG 450

G R D S K D N P E A M E E F K K F V K S K G F R E E N I T V  164
GGGAGAGACTCTAAGGACAACCCAGAGGCCATGGAGGAATTCAAGAAATTTGTAAAGAGCAAGGGATTCAGAGAAGAAAACATTACTGTC 540

P E L L D E C V P G S D *                                  176
CCTGAGCTGTTGGATGAGTGTGTACCTGGGAGTGACTAGGCACAGCTGCCCGTCAGGATAGAGTTGCTGATCCTGCCCTAATGCTGACTC 630
AGTTCTGATACATCCTGGGAGCTCCCGAACTCCAGACGACTTTCCTCACCTTCATGGATGGACTTCCCTTCCACCTCAGCTTCACCCACC 720
CCAGCACAGCTT                                                                              732
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RY2D1

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                *                                M T Q Q F W G P C L F S L F M  15
ACAGTTCCTGAGGTCTGAGACTCATCCAGTCTTCTTTGTACAACCATGACCCAGCAGTTCTGGGGTCCCTGTCTTTTCTCATTGTTTATG  90

A V L A Q E T L D P Q K S K V D C N K G V A G T V Y E Y G A  45
GCTGTATTGGCTCAGGAGACACTGGATCCTCAAAAATCAAAGGTGGATTGCAACAAAGGGGTGGCTGGCACCGTCTATGAGTACGGAGCC 180

N T L D G G E Y V Q F Q Q Y A G K H I L F V N V A S F C G L  75
AACACCTTAGATGGTGGGGAGTATGTCCAGTTCCAGCAGTATGCAGGAAAGCACATTCTCTTTGTCAACGTGGCGTCCTTCTGTGGCCTG 270

T A T Y P E L N T L Q E E L R P F N V S V L G F P C N Q F G  105
ACAGCTACGTACCCTGAACTGAACACATTGCAGGAGGAGCTGAGACCATTCAATGTCAGTGTTTTGGGCTTTCCATGCAACCAGTTTGGA 360

K Q E P G K N S E I L L G L K Y V R P G G G F V P N F Q L F  135
AAACAAGAACCTGGAAAGAACTCAGAGATCCTCCTTGGACTCAAATATGTTCGCCCAGGCGGTGGCTTTGTTCCCAATTTCCAGCTCTTT 450

E K G D V N G D N E Q K V F S F L K S S C P P T S E L L G S  165
GAGAAGGGGGATGTGAACGGAGACAATGAACAAAAGGTTTTTTCTTTCCTAAAGAGCTCCTGCCCTCCCACCTCTGAACTTCTTGGCTCT 540

P E H L F W D P M K V H D I R W N F E K F L V G P D G A P V  195
CCAGAACATCTCTTCTGGGATCCCATGAAGGTCCATGACATTCGCTGGAACTTTGAGAAGTTCCTGGTGGGACCGGATGGAGCCCCTGTC 630

M R W F H Q T P V R V V Q S D I M E Y L N Q T R T Q *      221
ATGCGCTGGTTCCACCAGACTCCTGTCAGAGTTGTCCAGTCAGACATCATGGAGTACCTAAACCAAACCCGTACCCAGTAGGATGTTTTA 720
CAAATAGTGCCTTTGCTCCACCCCTTCACCAGCCTCCATATGATCAAAGCCTGCAACACTACTTGTGTCCTTACTAATGTGTGTACAAAC 810
AGAAGTTTATTTGTGGTAGGCATATGCATGCATTGGAAATTAAGTTAAAAAAAAAAACAATTATCCACCCAGATATCTGACTCTCATATAT 900
CTAGTTTCCCTAATCTAGTCCAAAAGAAAGTCATATTTTGGCAATGATGATCCAGACAATTCCATATATCTAAAAAGTGATCTACCAAGA 990
CCCTAATCTATCATTCACAGCACTGGTGGGTTAAAAGAAGTGGGAACGGGTGGCCAGATCCTCTGGGTCCCAGTATCTTCTCCTCCCAAG 1080
CTGGGCATGCTTTGTCAAGCTCTCCTTCTGTTTAGCTCCTTAGTACAACACTGACCCATAGTTATCTTAGG                    1151
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FIGURE 3: Nucleotide sequences of RY2G12 and RY2D1 cDNAs and deduced amino acid sequences. The amino acid sequences are in single-letter code. The putative hydrophobic leader sequence of RY2G12 is underlined. The first ATG triplet of the indicated open reading frames for RY2G12 and RY2D1 most likely define the true translation initiation sites as each is preceded by an in-frame termination codon indicated by asterisks.

cDNAs, maximal homology alignment yielded 10.1% identity between OBP and RY2G12 and 8.1% identity between OBP and VEG, whereas RY2G12 and VEG exhibit 53.4% sequence identity (data not shown).

The second cDNA clone, RY2D1, is quite distinct from RY2G12 and does not appear to be a secretory molecule. It does, however, possess a significant level of homology to the enzyme glutathione peroxidase (GSHPx), with 49.2% identity between the amino acid sequences of the predicted RY2D1 polypeptide and rat GSHPx in a 189 amino acid overlap (Figure 4b). If conservative amino acid substitutions are considered, the overall homology is 82%. Within the coding region, the nucleic acid sequences were similarly homologous with 45.1% identity in a 514 nucleotide overlap (data not shown).

Discussion

Odorant Discrimination via OBPs Prior to Receptor Recognition. This study characterizes two cDNA clones corresponding to mRNAs which appear to be exclusively expressed in the OM. Their high level of expression in a tissue of such a highly specialized nature suggests a role in olfaction. One of these clones, OBP_{II}, encodes a secretory protein capable of binding odorants and is synthesized in the lateral nasal gland,

a

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RY2G12  MKSRLLTVLLLGLMAVLKAQEAPP-DDQEDFSGKW---Y--TKATVCD-R-NHTDGK-RPMKVFPMTVTA  61
VEG     MKALLLT-FGLSLIAALQAQAFPTTEENQDMSGTW---Y--LKAAAWD-K-EIPDKKFGSVSVTPMKIKT  62
OBP     MVKFLLIVLALGVSCA--HHENLD-ISPSEVNGDWRTLY--IVADNVE-K-VAEGG---SLRAY---FQH  57
BG      IRIIAIVVLFFLQCQADLPPVMKG-LEENKVTGVW---Y--GIAAASNCK-QFLQMK-SDNMPAEVNIYS  63
HC      LSACLAVSAGPVPTPPDNIQVQEN-FNISRIYGKW---YNLAIGSTCP-WLKKIMDRMTVST-LVLGEGA  74

RY2G12  LEGGD--LEVRLTFRGK--GHCHLRRITMHKTDEPGKYTTFKGKKTFYTKETPVKDHY----IF---YIK  120
VEG     LEGGN--LQVKFTVLIA--GRCKEMSTVLEKTDEPAKYTAYSGKQVLYIIPSSVEDHY----IF---YYE  121
OBP     MECGDECQELKLIFNVKLDSECQTHTVVGQKHED-GRYTTDVSGRNYF-HVLKKTDDI----IF---FHN  118
BG      LNNGH--MKSSTSFQTE--KGCQQMDVEM-TTVFKGHY-KWKMQQGDSETITVATDYD----AFLMEFTK  123
HC      TE-AE--ISMTSTRWRK--GVCEETSGAYEKTDTDGKFLYHKSKWNITMESYVVHTNYDEYAIF---LTK  136

RY2G12  G-QRHGKSYLKGKLV-GRDSKDNPEAMEEFKKFVKSKGFREENITVPELLDECVPGSD              176
VEG     G-KIHRHHFQIAKLV-GRDPEINQEALEDFQSVVRAGGLNPDNIFIPKQSETCPLGSN              177
OBP     V-NVDESGRRQCDLVAGKREDLNKAQKQELRKLAEEYNIPNENTQHLVPTDTCNQ                  172
BG      I-QMGAEVCVTVKLF-GRKDTLPEDKIKHFEDHIEKVGLKKEQYIRFHTKATCVPK                 177
HC      KFSRHHGPTITAKLY-GRAPQLRETLLQDFRVVAQGVGIPEDSIFTMADRGECVPGEQ               193
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b

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RY2D1   KSKVDCNKGVAGTVYEYGANTLDGGEYVQFQQTAGKHILFVNVASFCGLTA-TYPELNTLQEELRPFNVS   95
                .:::..:..:.::: : .    :: .:. :::::..: :. .:.:.:.::. : : ..
GSHPx   MSAARLSAVAQSTVYAFSARPLAGGEPVSLGSLRGKVLLIENVASLCGTTTRDYTEMNDLQKRLGPRGLV   70
                                                *
RY2D1   VLGFPCNQFGKQEPGKNSEILLGLKYVRPGGGFVPNFQLFEKGDVNGDNEQKVFSFLKSSCPPTSE----   161
        :::::::::::.:: :::.::: .:::::::::: ::: :::: .::::... .:.:::.. :..::
GSHPx   VLGFPCNQFGHQENGKNEEILNSLKYVRPGGGFEPNFTLFEKCEVNGEKAHPLFTFLRNALPAPSDD PTA   140

RY2D1   LLGSPEHLFWDPMKVHDIRWNFEKFLVGPDGAPVMRWFHQTPVRVVQSDIMEYLNQTRTQ             221
        :...:...:.:.  .:::::::::::::::.::.:. .. ..  ...:: . :.. ...
GSHPx   LMTDPKYIIWSPVCRNDISWNFEKFLVGPDGVPVRRYTRRFRTIDIEPDIEALLSKQPSN            200
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FIGURE 4: Alignment of the predicted protein sequences for RY2G12 and RY2D1 with homologous proteins. (A) Predicted RY2G12 protein aligned with rat VEG protein (Schmale et al., 1990), rat odorant-binding protein (Pevsner et al., 1988b), frog olfactory precursor protein BG (Lee et al., 1987), and human α_1-microglobulin (Kaumayer et al., 1986). Amino acids in common with RY2G12 are boxed. Gaps, indicated by dashes, were introduced to maximize alignment. (B) Alignment of rat GSHPx (Ho et al., 1988) and RY2D1 protein sequences. Double dots indicate identical amino acids in both proteins, while single dots indicate conservative amino acid substitutions. The position of the selenocysteine residue in GSHPx is indicated by an asterisk below the cysteine residue.

which is the site of synthesis of the described OBP (Pevsner et al., 1988a). This protein is localized to the mucus layer, and its ability to bind odorants argues for a role in olfaction. The lack of odorant-binding specificity (Pelosi & Tirindelli, 1989; Pevsner et al., 1990) and its secretory nature (Pevsner et al., 1988b), however, suggest that it is not a "receptor" molecule involved in odorant recognition (Pelosi & Tirindelli, 1989). OBP probably participates in olfaction by binding to hydrophobic odorants and transporting them across the mucus layer to the receptor sites. Alternatively, these proteins may act as "scavengers", removing odorant from receptors once they have been stimulated (Schofield, 1988).

The finding of an OBP homologue suggests that differential odorant specificity may exist for OBP and OBP_{II}, which may contribute to odorant recognition prior to interaction with ciliary receptors. The significant divergence exhibited by OBP and OBP_{II} intimates that the two proteins may possess different ligand affinities and specificities. In addition, other OBP family members may exist (Vogt et al., 1991), and the differences between OBP and OBP_{II} suggest the possible existence of distinct receptor classes for the OBPs in the cilia of the olfactory epithelium.

Odorant Removal in the Olfactory Mucosa. The RY2D1 DNA sequence predicts a nonsecretory protein. The sequence has high homology to rat glutathione peroxidase (GSHPx), a cytosolic and mitochondrial enzyme that catalyzes the reduction of a variety of peroxides, utilizing glutathione as the reductant substrate (Flohe, 1982). It is believed to play a crucial role in protecting cell membranes and DNA from peroxide-mediated damage (Sandstrom & Marklund, 1990). Several different forms of the enzyme have been identified (Duan et al., 1988; Maddipati et al., 1987; Ursinin et al., 1985) and cloned (Akasaka et al., 1990; Chambers et al., 1986; Dunn et al., 1989; Ho et al., 1988). An unusual feature of these sequences is that a selenocysteine residue in the active site of the protein is encoded by the nonsense codon UGA. The corresponding codon is UGU in the RY2D1 sequence, resulting in the replacement of selenocysteine with cysteine in the amino acid sequence. This selenocysteine is an essential constituent of the active site of GSHPx (Forstrom et al., 1978), implying that RY2D1 is not merely an OM-specific form of GSHPx but may have differing enzymatic specificities.

The striking level of similarity between the RY2D1 and GSHPx protein sequences suggests that RY2D1 may be involved in some aspect of detoxification. The mammalian olfactory mucosa has been demonstrated to possess high levels of activity for a variety of drug-metabolizing enzymes (Dahl, 1988). Unique forms of UDP-glucuronyl transferase (UGT_{olf}) and cytochrome P450, both of which are involved in detoxification pathways, have been identified in the OM (Lazard et al., 1990; Nef et al., 1989), and like RY2D1, both enzymes are expressed in the Bowman's glands (Lazard et al., 1991; Zupko et al., 1991). The Bowman's glands may, therefore, possess a battery of detoxification enzymes which enable a broad spectrum of odorants to be targeted for biotransformation.

It has been proposed that olfactory-specific detoxification enzymes may terminate diverse odorant signals, enabling new signals to be received (Lazard et al., 1991). The primary termination signal in olfactory transduction is most likely initiated at the receptor site itself by removal of the odorant from the receptor at the cilia surface. The predicted RY2D1 protein is unlikely to be secreted from the Bowman's glands and, therefore, would not contribute to such a process. The

expression of presumptive detoxification enzymes in the subepithelial Bowman's glands suggests that they could encounter odorants which are present in the mucus layer but have not accessed ciliary receptor sites. Consequently, these enzymes may contribute to the clearance of such odorants from the neuroepithelium, thereby preventing the initiation of new olfactory signals from residual odorant molecules. Alternatively, these enzymes may act as a primary defense against potentially harmful odorants by metabolizing them into less harmful compounds.

Odorant Discrimination Mechanisms. The recently identified odorant receptor proteins (Buck & Axel, 1991) most likely account for the majority of the discriminating ability of the olfactory system. However, the identification of divergent forms of rat odorant-binding protein suggests that such proteins may contribute to vertebrate odorant recognition and discrimination. The mammalian olfactory system may, therefore, possess the ability to discriminate odorants at various levels. First, within the mucus layer the diverse OBPs recognize and bind distinct classes of odorants. Second, the putative ciliary receptors may discriminate between individual odorants, and finally, the detoxification enzymes of the Bowman's glands may target specific classes of odorants for degradation.

ACKNOWLEDGMENTS

We thank T. Boehm for help with in situ hybridization, B. Keverne for discussions, and M. Kennedy for critical reading of the manuscript. We also thank J. Fogg and T. Smith for oligonucleotide synthesis, G. King and T. Langford for assistance with laboratory animals, and the photography department for assistance with preparation of figures.

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An Unusual Peptide Conformation May Precipitate Amyloid Formation in Alzheimer's Disease: Application of Solid-State NMR to the Determination of Protein Secondary Structure†

Richard G. S. Spencer,‡,‖ Kurt J. Halverson,§ Michèle Auger,‡ Ann E. McDermott,‡ Robert G. Griffin,‡,§ and Peter T. Lansbury, Jr.*,§

Department of Chemistry and Francis Bitter National Magnet Laboratory, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, and Laboratory of Cellular and Molecular Biology, Gerontology Research Center, National Institute on Aging, National Institutes of Health, Baltimore, Maryland 21224

Received June 28, 1991; Revised Manuscript Received August 19, 1991

ABSTRACT: The formation of insoluble proteinaceous deposits is characteristic of many diseases which are collectively known as amyloidosis. There is very little molecular-level structural information available regarding the amyloid deposits due to the fact that the constituent proteins are insoluble and noncrystalline. Therefore, traditional protein structure determination methods such as solution NMR and X-ray crystallography are not applicable. We report herein the application of the solid-state NMR technique rotational resonance (R^2) to the accurate measurement of carbon-to-carbon distances in the amyloid formed from a synthetic fragment (H_2N-LeuMetValGlyGlyValValIleAla-CO_2H) of the amyloid-forming protein of Alzheimer's disease (AD). This sequence has been implicated in the initiation of amyloid formation. Two distances measured by R^2 indicate that an unusual structure, probably involving a cis amide bond, is present in the aggregated peptide amyloid. This structure is incompatible with the accepted models of fibril structure. A relationship between this structure and the stability of the amyloid is proposed.

Amyloid deposits are characteristic of many diseases (Castaño & Frangione, 1988; Glenner, 1980a,b; Stone, 1990; Goate et al., 1991) including Alzheimer's disease (Selkoe, 1990; Glenner, 1988) and type II diabetes (Nishi et al., 1990). The deposits, or plaques, comprise protein fibrils which share affinity for certain dyes (Cooper, 1974) and a regular, repeating structure (Crowther, 1991). The naturally derived amyloid plaque from AD[1] brain has been analyzed by X-ray fiber diffraction and produces a distinctive pattern of reflections (Kirschner et al., 1986). This diffraction pattern was observed for *Bombyx mori* silk (Marsh et al., 1955) and for polyalanine (Pauling & Corey, 1953b) and gave rise to Pauling's widely accepted model of a cross-β fibril (see Figure 1) (Marsh et al., 1955). The cross-β fibril model consists of antiparallel peptide chains which are arranged perpendicular to the direction of fibril growth, to form a β-pleated sheet. The strands in each β-sheet interact via a network of interstrand hydrogen bonds (Marsh et al., 1955; Arnott et al., 1967). Sheets are stacked in a parallel fashion, the intersheet distance being governed by the identity of the amino acid side chains (Marsh et al., 1955; Geddes et al., 1968). Although lamellar silk crystals have been grown (Lotz et al., 1982), single crystals suitable for crystallographic studies have not been produced; hence, the details of the cross-β fibrillar structure and its constituent antiparallel β-sheet have not been elucidated. This information is required in order to fully understand the factors which direct amyloid formation and, more generally, the sequence dependence for β-sheet formation (von Heijne & Blomberg, 1977; Lifson & Sander, 1980).

The synthetic nine amino acid peptide H_2N-LMVGGVVIA-CO_2H (β34–42) (Halverson et al., 1990) represents the carboxy-terminal portion (residues 34–42) of the 42 amino acid β/A4 protein. The β/A4 protein is the major constituent of the extracellular amyloid plaque that characterizes the brains of victims of AD and advanced Down's syndrome (Masters et al., 1985). The 34–42 portion of the β/A4 sequence is thought to be part of the transmembrane sequence in the amyloid precursor protein (Selkoe, 1990; Glenner, 1988). Proteolytic excision of the β/A4 sequence from the precursor protein may be characteristic of the disease state (Sisodia et al., 1990). The β34–42 peptide is extremely insoluble and readily forms cross-β fibrils which can be observed by electron microscopy (Halverson et al., 1990). This

†This work was supported by the NIH (AG08470 to P.T.L. and GM23403 and RR00995 to R.G.G.), Alzheimer's Disease Research (a program of the American Health Assistance Foundation, Rockville, MD; to P.T.L.), the Whitaker Health Sciences Fund (P.T.L.), the NSF (Presidential Young Investigator Award to P.T.L.), and Merck & Co. (Faculty Development Grant to P.T.L.). R.G.S.S., M.A., and A.E.M. were supported by postdoctoral fellowships from the NIH (GM13557), the Natural Sciences and Engineering Research Council of Canada, and the American Cancer Society (PF3283), respectively.

‡Francis Bitter National Magnet Laboratory, MIT.
§Department of Chemistry, MIT.
‖National Institute on Aging, NIH.

[1] Abbreviations: R^2, rotational resonance; AD, Alzheimer's disease; FABMS, fast atom bombardment mass spectrometry; MM, molecular mechanics; ssNMR, solid-state NMR; CPMAS, cross-polarization magic-angle spinning.

0006-2960/91/0430-10382$02.50/0