Structure of a Specific Alcohol-Binding Site Defined by the Odorant Binding Protein LUSH from Drosophila Melanogaster

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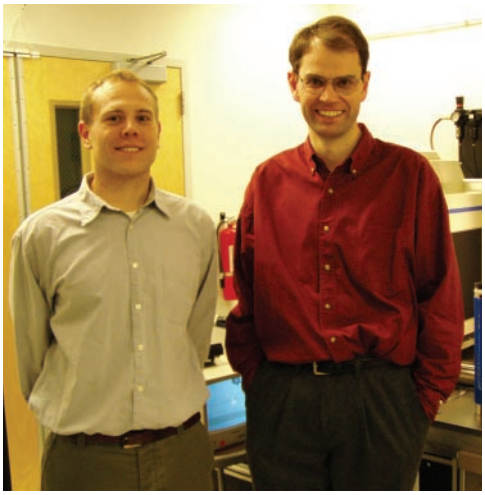
LUSH is a non-enzymatic alcohol-binding protein that is required for a normal chemosensory response to alcohols, such as ethanol in the fruit fly Drosophila melanogaster. We have recently solved the structures of LUSH in complex with ethanol, n-propanol, and n-butanol to 1.49 angstroms (Å), 1.45Å, and 1.25Å, respectively. From these structures we have identified a set of inter-molecular interactions that act in concert with each other and may help to increase the binding affinity of alcohol to the protein, and thereby defines a specific alcohol-binding site. We propose that this structure may represent a general alcohol-binding motif that is believed to exist in a number of alcohol-sensitive ion channels in mammalian cells.

There is substantial evidence that many of the biochemical effects of alcohol are linked to changes in the structure and dynamics of a number of receptors in the central nervous system, including the gamma-amino butyric acid (GABA) and nicotinic acetylcholine (nAChR) receptors. Alcohol appears to bind to these receptors at specific sites, but the details of this interaction remain unclear because of the lack of structural information about these membrane-associated proteins. Our goal is to determine the molecular architecture of specific alcohol-binding sites through structural studies of a model protein, LUSH, from the fruit fly Drosophila melanogaster. In insects, odorant binding proteins are key components of the insect sensory system and bind to odor molecules from various sources, including those from food or other insects. LUSH is an odorant binding protein that is required for the normal behavioral response to alcohols. Adult flies have an active avoidance mechanism that responds to high concentrations of short chain alcohols, but this mechanism is defective in flies that lack the lush gene. LUSH was given its name because fruit flies without the corresponding gene are attracted to alcohol.

From the high-resolution structures of LUSH determined by Miyazawa et al., we identified a structural motif that may provide a high-affinity alcohol-binding site. An essential part of this study was the availability of the "Mail-in Data Collection" program at the NSLS. Since we work in a laboratory that traditionally uses nuclear magnetic resonance (NMR) spectroscopy to analyze protein structures, this service gave our group rapid access to synchrotron beam time, which would have otherwise been difficult to obtain through a conventional application. In addition, we received expert assistance from NSLS staff, in particular Dr. Howard Robinson. LUSH protein labeled with



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seleno-methionine was crystallized at the University of Colorado Health Sciences Center and sent to the NSLS for data collection. As a result, we were able to solve the structure in a matter of weeks, rather than several months.

The structure of LUSH consists of six α-helices and a short segment of a 3_{10}-helix that surround a central cavity that is hydrophobic (does not like aqueous environments) and contains the alcohol-binding site. The hydroxyl group of the alcohol forms hydrogen bonds (H-bonds) to two amino acids in LUSH, serine (Ser52) and threonine (Thr57), at one end of this cavity (**Figure 1**). The structure was solved in the presence of 30 to 50 millimolar (mM) alcohol, and under these conditions we only observe the one alcohol-binding site, suggesting that the chemistry within this site may have evolved to specifically bind alcohols. We hypothesize that this is a result of the side-chain hydroxyl of Ser52 making an H-bond to the main-chain oxygen of Thr48, another amino acid, increasing the ability of the side chain of Ser52 to accept a H-bond from the alcohol. The positioning of Thr57 as a H-bond donor to the alcohol may simultaneously make the alcohol a better H-bond donor to Ser52.

The recent structure of the nAChR from the Torpedo Ray suggests the possibility that serine and threonine residues could be positioned within the protein interior to make a set of concerted interactions with alcohol in a manner analogous to that seen in LUSH. If so, then as the acetylcholine receptor is the archetypal model for several receptors implicated in the effects of alcohol consumption. We suggest that the alcohol-binding site defined in LUSH may represent a general model for alcohol binding in other proteins. We are now testing the role that individual amino acids play in binding alcohol by solving the structure of proteins that have undergone amino acid substitutions. We believe these structures will provide further insights into the nature of alcohol-binding sites.

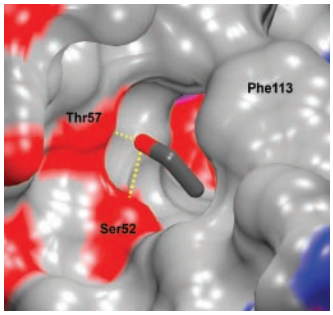

Figure 1. A surface representation of key residues in the LUSH alcohol-binding pocket. The pocket is lined with hydrophobic residues with the exception of Thr57 and Ser52, which form concerted hydrogen bonds with the alcohol. Ethanol is shown in a stick representation. The hydrogen bonds between the alcohol and the hydroxyl groups of Thr57 and Ser52 are shown as yellow dotted lines.