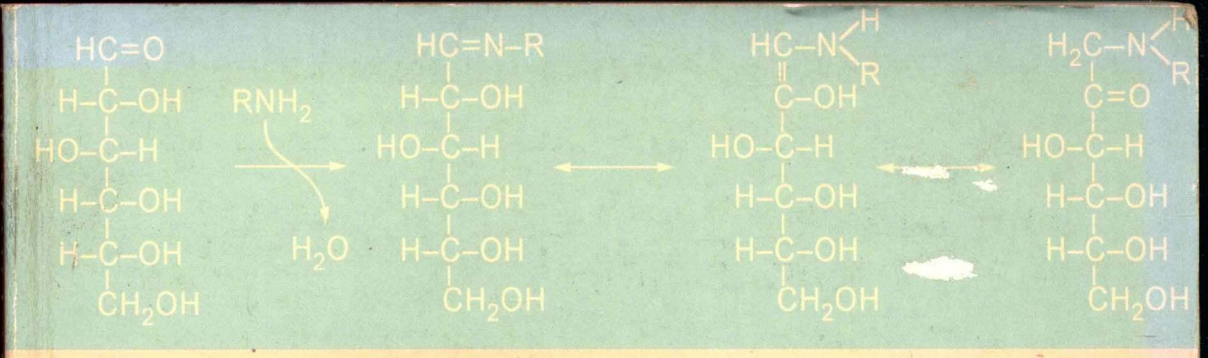

The Maillard Reaction

Recent Advances in Food and Biomedical Sciences

edited by

Erwin Schleicher

Veronika Somoza

Peter Schieberle

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Volume 1126

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ERWIN SCHLEICHER, VERONIKA SOMOZA, AND PETER SCHIEBERLE

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Preface

The 9th International Symposium on the Maillard Reaction, held in Munich in September of 2007, offered a wealth of stimulating talks and discussions. More than 200 delegates from academia and industry and from more than 20 nations actively contributed to the success of the symposium. Following earlier trends, the interdisciplinary nature of the meeting was impressively emphasized by the wide range of subjects addressed. These encompassed food science, food technology and processing, and toxicology of processed food, as well as taste, flavor, and satiety-sensing mechanisms. In food science, the majority of contributions circled around the elucidation of the molecular mechanism of the Maillard reaction, the identification of adverse pathways and their mitigation. In the biomedical field, the interest focused on the development of diabetic complications, particularly affecting the eye, nerves, kidneys, and also the macrovascular system, and on the pathogenesis of neurodegenerative diseases. Moreover, several contributions provided evidence for the involvement of the Maillard reaction products/advanced glycation end products (AGEs) and their receptors in the immune system, in cancer, and, unexpectedly, in anxiety. The improvements in new analytical techniques, particularly chromatography coupled with mass spectrometry, greatly enhance the sensitivity and specificity of analysis, leading to the characterization of new Maillard reaction products in food and biological samples and, thus, potentially leading to the identification of new biomarkers for associated diseases.

Because no other international conferences encompass the scope of the Maillard symposium and because the area is spreading very rapidly, it has been decided that future meetings will take place biannually. The next location will be Australia for the meeting in 2009, and the 2011 symposium will be held in France in honor of the 100th anniversary of Louis Camille Maillard's first publication describing the "browning reaction" that now carries his name.

We also wish to express our sincere appreciation to the local organizing committee members, as well as to the international advisory board and all other contributors. We especially thank our sponsors for their generous support in helping to make this symposium successful (list is in alphabetical order): Deutsche Forschungsanstalt für Lebensmittelchemie, DiagnOptics, Firmenich, Gambro, the German Research Foundation (DFG), the German Society of Clinical Chemistry and Laboratory Medicine (DGKL), the International Maillard Reaction Society, L'Oreal, Nationales Aktionsforum Diabetes mellitus (NAFDM), the National Institute of Diabetes and Digestive and Kidney Diseases, Nestlé, NeoMPS, Procter & Gamble, Roche, the Technical University of Munich, and the University Clinic of Tübingen.

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The Maillard Reaction

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This volume is the result of the **9th International Symposium on the Maillard Reaction**, held September 1–5, 2007 in Munich, Germany.

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Part III. Oral Presentations

The Sense of Smell

Reception of Flavors

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The sensory and hedonic evaluation of most food-related flavors is mainly dependent on olfactory perception. The sense of smell is able to recognize and discriminate myriads of airborne molecules with great accuracy and sensitivity. The primary processes of odor perception are mediated by the chemosensory olfactory neurons in the nasal epithelium, which upon interaction with appropriate odorants elicit a chemo–electrical transduction process converting the chemical signal into electrical impulses. The encoded information is conveyed onto distinct glomeruli, inducing topographic activity patterns in the olfactory bulb. The emerging chemotopic maps are decoded in the olfactory cortex, leading to the perception of distinct flavors.

Key words: **olfaction; olfactory neurons; odorant receptor; signal transduction; axonal wiring; odor coding; information processing**

Introduction

The flavors for most of our food and beverages are generated during processing of raw materials, such as roasting, cooking, and baking, when chemical reactions not only produce the characteristic brown and golden colors but also produce a large number of odorous compounds. Especially the nonenzymatic browning reaction—the Maillard reaction—leads to the formation of multiple volatile compounds, thus creating the characteristic flavor of foods and beverages.[1] Maillard reaction products are formed when, under high temperature conditions, reducing sugar molecules react via their carbonyl group with the NH_2 group of amino acids. Since the amino acid types determine the flavor of the resulting compounds, the diversity of proteins and sugars in foods accounts for literally hundreds of odorous compounds, creating the characteristic flavor of roasted coffee, grilled meat, toasted bread, fried onions—to mention a few. The Maillard products determine the profoundly different flavors of, for example, meat cooked in boiling water compared to meat cooked in a fryer at a temperature above 120°C.[2] A precise correlation between the composition of Maillard products in processed foods and the human perception is still elusive. However, it can be assumed that most of the compounds are sensed by the nose. In fact, it has been estimated that in approximately 80–90% of what is perceived as food, "taste" actually is due to the sense of smell.

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Process of Olfaction

Our nose has an enormous capacity to recognize and discriminate myriads of small volatile compounds of many chemical classes and structural diversity, ranging from short-chain aliphatic compounds to complex aromates with multiple side chains and functional groups. In fact, even novel compounds, which were designed and synthesized by chemists, are immediately recognized by the olfactory system and perceived as distinct odor. Moreover, the nose is also a very sensitive chemodetector; it recognizes odorous compounds at concentrations as low as a few parts per trillion.[3] An understanding of the mechanisms underlying the remarkable sensory capacity of the nose, the principle for encoding the complex sensory information, as well as how the brain reconstructs these stimuli into a "smell map" of the world is a major objective for research in olfaction and has greatly advanced over the past decades.[4,5] The perception of an olfactory stimulus is accomplished by two main processes: the primary signal transduction events in the nasal neuroepithelium and the processing of sensory information in the olfactory bulb and higher brain centers. The process of olfactory perception begins when inhaled odorous compounds dissolve in the mucus that covers and protects the epithelium. Odorants are recognized by millions of chemosensory cells residing in the nasal neuroep-

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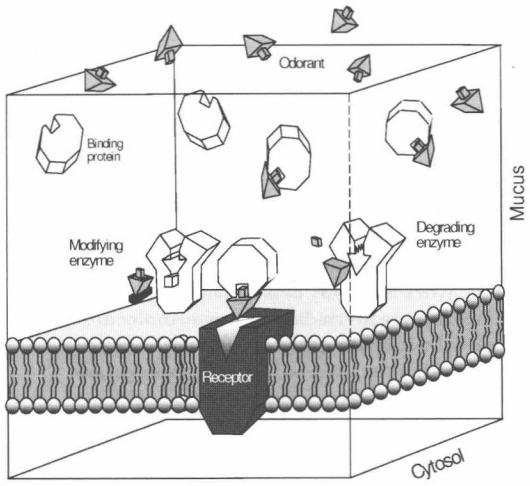

FIGURE 1. Processes in the mucus layer of the olfactory epithelium influence the entry, exit, or residence time of odorous molecules in the receptor environment. These ancillary processes are integral components of the chemical-sensing systems; they include the interaction with soluble-binding proteins, which may act as possible shuttles of the volatile, lipophilic, odorous molecules through the aqueous mucus layer, as well as the inactivation of odorants by degrading and/or biotransformation enzymes, thus clearing the system between consecutive sniffs. (From Breer.[31] Reproduced with permission.)

ithelium within the posterior cavity of the nose. The bipolar olfactory neurons send an axon to the olfactory bulb and extend a dendritic process to the nasal lumen. The tip of the apical dendrite carries 5–20 cilia that are embedded in the protecting nasal mucus and exposed to the external environment; they are the sites of primary olfactory events.

Perireceptor Processes

The airborne primarily lipophilic odorants must traverse the aqueous milieu of the mucus layer covering the nasal epithelium before contacting the olfactory cilia. The entry, exit, and residence time of lipophilic odorants in the receptor environment are considered an important part of the chemical-sensing process, although the mechanisms for these "perireceptor events" are still poorly understood (FIG. 1). The discovery of abundant, small, globular proteins in the mucus fluid surrounding the sensory dendrite and cilia, which are produced by the glands of the nasal cavity, has led to the concept that these odorant-binding proteins, which are members of the lipocaline family, may accommodate hydrophobic molecules in an aqueous environment and enhance their access to the receptor sites.[6] For a continuous monitoring of the chemical environment, a rapid inactivation and clearance of odorous molecules is necessary to maintain the capability of the olfactory system to receive iterative incoming signals with every breathing airstream. This seems to be accomplished by the action of biotransformation enzymes.[7] The reaction of phase I enzymes (e.g., cytochrome *P*-450 mono-oxygenases), which introduce chemical changes, such as hydroxylation, is followed by phase II enzymes, such as UDP-glucuronosyl transferase or glutathione-*S*-transferase catalyzing the conjugation of glucuronic acid or of glutathione to phase I-modified odorants. Odorous molecules modified by this sequential biotransformation are no longer lipid soluble and are incapable of receptor stimulation.

Chemo–electrical Transduction

Upon interaction of odorants with the chemosensory cilia of appropriate olfactory cells, the processes of chemo–electrical signal transduction are elicited, leading to an inwardly depolarizing current that is converted to a distinct frequency of action potentials which are conveyed, via the axon, to the olfactory bulb and deciphered by higher brain centers.[8] Thus, the strength and duration of odorant stimuli are encoded into patterns of neuronal signals. In most of the ciliated olfactory sensory neurons, signal transduction seems to be mediated via the G_{olf}/adenylyl cyclase III (ACIII)/cyclic adenosine monophosphate (cAMP)-pathway (FIG. 2). The interaction of suitable odorants with distinct receptor proteins in the ciliary membrane activates trimeric G-proteins, which stimulate the specific ACIII, thus efficiently generating cAMP. This leads to a very rapid and transient increase in cAMP concentration with a time course of a few hundred milliseconds. Starting from a presumed micromolar concentration of cAMP, the observed five- to ten-fold increase brings the cAMP level well above the kd value determined for the cAMP-gated channels in the ciliary membrane. Thus, the elevated cAMP level triggers membrane depolarization via opening of cyclic-nucleotide-gated ion channels preferentially permeable for Ca^{2+} ions, which causes an elevation of intraciliary Ca^{2+} concentration.[9] In olfactory cilia, elevation of Ca^{2+} concentration activates ion channels that permeate chloride ions. Interestingly, the calcium-activated chloride conductance further depolarizes the cell. This untypical reaction is based on the unusually high intracellular Cl^- concentration in olfactory neurons, leading to an efflux of Cl^- ions through Ca^{2+}-activated-chloride channels.[10]

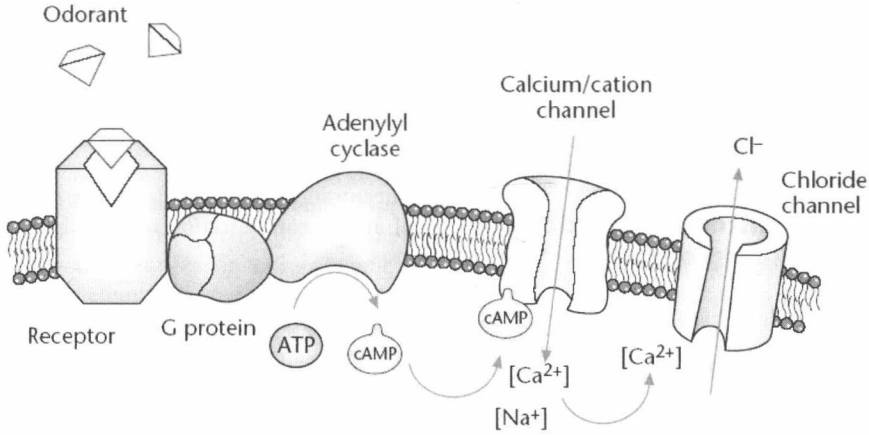

FIGURE 2. Schematic representation of the chemo–electrical transduction pathway in olfactory sensory neurons. Upon binding of appropriate odorous ligands, odorant receptors in the ciliary membrane act through specific G-proteins (G_{olf}) to stimulate adenylyl cyclase (type III) generating cAMP. The resulting elevated second-messenger levels elicit the activation of cation channels, allowing the influx of sodium and especially calcium ions. Calcium ions, in turn, activate Ca^{2+}-dependent chloride channels. Because of the characteristic equilibrium potential for chloride in olfactory neurons, the induced Ca^{2+} current is depolarizing, thus resulting in a significant amplification of the primary odor-induced current. (From Breer.[31] Reproduced with permission.)

The Ca^{2+}-mediated linkage from cyclic-nucleotide-gated channels to chloride channels has been considered a mechanism that contributes to the high-gain and low-noise amplification of olfactory neuron responses. The transduction cascade is reset by terminating the formation of cAMP via feedback reactions including receptor phosphorylation, GTPase activity of Gα subunits, Ca^{2+} block of CNG channels, and by hydrolysis of cAMP in 5′-AMP catalyzed by phosphodiesterase. The influx of calcium via the cAMP-gated channels is also important for the process of olfactory adaptation, i.e., decrease of a stimulus perception upon stimulation over longer periods, which appears to be a result of a decaying responsiveness of olfactory sensory neurons. Calcium ions entering the olfactory cilia interact with calmodulin, and the newly formed calcium–calmodulin complex closes the ion channel even in the presence of cAMP.[11] This negative-feedback loop turns off the electrical response of an olfactory neuron even in the presence of appropriate odorants. This mechanism is supplemented by kinase reactions, leading to phosphorylation of transduction elements and thereby inducing longer lasting desensitization effects.

Olfactory Receptors

How thousands of different odorants which vary widely in size and structure are readily detected and discriminated has been a long-standing puzzle. The accuracy of odor discrimination depends on the specificity with which odorants activate olfactory neurons via distinctive receptor proteins residing in the ciliary membrane. Thus, olfactory receptor proteins can be considered as molecular entities at the interface between the environment and the nervous system. Accordingly, understanding the nature, diversity, and specificity of receptors for odorants has always been considered key for understanding the molecular basis of olfaction. The discovery of a large family of genes, which encode heptahelical transmembrane proteins and are expressed in the olfactory epithelium,[12] was the ground-breaking work for a wealth of studies indicating that odorant receptors (ORs) are members of the G-protein-coupled receptor superfamily; they are encoded by a multigene family comprising as many as a thousand distinct genes that are organized in clusters at many different loci spread over all but a few chromosomes in the mouse genome. In the human genome, about 900 OR genes have been identified, but two-thirds of these turned out to be nonfunctional or "pseudogenes," which have lost their function during evolution; a total of 347 putative, functional OR genes in the human genome was determined.[13] In spite of the reduced number of OR subtypes, the human olfactory system retained the ability to recognize a broad spectrum of chemicals; however, its discriminatory capacity is probably reduced compared to mice. The high proportion of pseudogenes[14] and the unusually high rate of single nucleotide polymorphisms in hu-

man receptor genes[15] indicate a variable repertoire of functional OR genes in the human population. Many specific anosmia (i.e., the inability to smell particular odors) could be a result of hereditary defects of OR genes.

Response Specificity of an Odorant Receptor

Physiological recordings have demonstrated that individual olfactory sensory neurons typically respond to a variety of different odorants and that each cell shows a unique order of agonist potency, indicating that olfactory neurons are highly diverse and broadly tuned.[16,17] Based on the notion that each olfactory sensory cell expresses only one OR subtype, it seems likely that a relatively nonspecific ligand spectrum is a characteristic feature of ORs. The assessment of the responsiveness of a distinct receptor type requires the expression in heterologous cells. The problems encountered in this approach were circumvented by an *in vivo* expression system transfected by means of recombinant adenovirus and assessed by electrophysiological recordings[18] or by *in vitro* systems using engineered OR chimeric receptors.[19] The general consensus from these studies is that a distinct OR type is activated by multiple odorants and that the range of dissimilar ligands for a distinct OR subtype resembles that of individual olfactory sensory neurons.[20] Not only can a distinct receptor type recognize multiple odorants but, at the same time, a single odorant is capable of activating multiple receptor types. Thus, all data point to the concept that the nose uses a combinatorial coding scheme to discriminate the vast number of different smells. Analogous to the visual system, which uses three receptor types (three opsin subtypes of the three-cone populations) to make sense of all perceivable colors, the olfactory system computes information from combinations involving any of hundreds of OR types. The numerous possible combinations explain the capacity of the system to encode an unlimited number of odors. Instead of dedicating an individual odor receptor to a specific odor, the olfactory system uses an "alphabet" of receptors to create a specific odor response[21]; in this view, a distinct receptor type participates in encoding very different odors, much the same way as a distinct letter participates in forming very different words. The principle of combinatorial coding implies that odorants of nearly identical structure are recognized by different but overlapping sets of receptors, thus explaining why even a slight change in the structure of an odorant can cause a dramatic shift in its perceived odor; for example, when the hydroxyl group of octanol is replaced by a carboxyl group to make octanoic acid, its odor changes from orange to rancid. Combinatorial coding may also explain the phenomenon that the perceived quality of an odorant can differ with a change in its concentration; for example, indole is perceived as floral at low concentration but smells putrid at higher doses.

Spatial Expression Patterns of Odorant Receptors

Despite the large number of receptor genes, a given sensory neuron seems to express only one type of receptor derived from a single allele. This notion implies that the supposedly about 20 million olfactory neurons of a mouse can be subdivided into about a thousand subpopulations. Within the olfactory epithelium, subpopulations (i.e., groups of 10,000–20,000 olfactory sensory neurons expressing the same receptor type) are confined to one of several broad expression zones. Within a zone, the neurons expressing a distinct receptor type are broadly distributed and surrounded by cells expressing different receptor types.[22,23] The functional implications of this zonal segregation are still elusive, but it is maintained in the olfactory bulb with each zone of the epithelium projecting to a distinct region of the olfactory bulb. In contrast to the zonal distribution of most subpopulations, olfactory sensory neurons expressing receptor subtypes of the OR37 subfamily are segregated in a small area on the tip of central turbinates spatially organized in clusters.[24] The functional relevance of this unique distribution pattern is unknown, but it is maintained in a distinct projection area in the bulb.[25]

Projection Patterns of Olfactory Neurons

To inform the brain, each olfactory neuron projects a single unbranched axon into the olfactory bulb where it terminates in rounded regions of neuropil, termed *glomeruli*, which are considered anatomical and functional units for olfactory processing (FIG. 3). This spherical neuropil structure of about 100 μm in diameter and surrounded by glia cell processes consists of the arborizing preterminal fibers and terminal boutons of the olfactory axons synapsing onto the distal dendritic tufts of the mitral cells as well as the periglomerular neurons. Thousands of olfactory neurons project their axon onto one glomerulus, thus achieving a high