Reports

Chemical Sensing: An Approach to Biological Molecular Mechanisms Using Difference Spectroscopy

Abstract. *Stimulation of preparations of rabbit olfactory epithelium with aqueous solutions containing the odorants linalool or inalyl isobutyrate, or both, initiates molecular changes that can be monitored by a specific decrease in absorbance at 267 nanometers. This change in absorbance appears to be associated with a change in macromolecular conformation which is initiated by formation of a complex involving the stimulant molecules.*

Chemically induced changes in preparations from rabbit olfactory mucosa were monitored by means of the ultraviolet difference spectroscopy technique. This technique has been used by others to follow changes in molecular conformation (*1*).

The findings of Dastoli and Price (*2*), in which they reported sugar-induced changes in the ultraviolet absorbance of protein preparations from taste buds, suggest that this technique might also be utilized to investigate the molecular mechanisms of the olfactory receptors. In my work the ultraviolet difference spectra have been routinely monitored from 450 to 250 nm by use of the Cary recording spectrophotometer, model 14; no changes in absorbance have been found in the visible region beyond 450 nm. An interesting time-dependent decrease in absorbance at 267 nm was consistently observed when preparations I used were stimulated with aqueous solutions saturated with the floral odorant linalool.

The chemical stimulant, linalool, was selected from the recommended odor list prepared by an ad hoc committee on olfaction, set up at the 1966 Gordon Conference to study osmics for sensory research. However, samples of linalool from two suppliers resulted in quite different activity levels at 267 nm. Gas chromatographic analysis (*3*) of these two samples showed that the most active one contained impurities that were present at less than 0.1 percent of the supplier's sample of linalool. The major impurities were identified (*3*) and their effect on the preparations of olfactory mucosa were determined. Only two compounds, linalool and linalyl isobutyrate, caused a change in absorbance at 267 nm; when these two compounds were present in the same solution they had a synergistic effect on the activity at 267 nm. The solution of stimulant that was used for the experiments described in this report contained $0.01M$ linalool and $<1 \times 10^{-5}M$ linalyl isobutyrate. The activity at 267 nm was highly specific (this subject will be discussed in a report to be published later).

The data from one experiment, as obtained from the recording spectrophotometer with the use of the 0 to 0.1 absorbance slide wire, are presented in Fig. 1, which shows the general shape of the response curve with its maximum change in absorbance at 267 nm. The lower or base line trace is the difference spectra recorded with the biological preparation in both chambers just prior to addition of the chemical stimulant. Any absorbance due to the stimulant was balanced out by including the stimulant in the light path of both chambers; in the reference chamber the stimulant was mixed with the biological preparation, while in the sample chamber it was placed at the same concentration in a second quartz cell containing buffer. As the reaction between the biological preparations and the stimulant proceeded the change in absorbance at 267 nm became more distinct.

Recently conformational differences between biologically active and inactive forms of a transfer ribonucleic acid were reported (*4*). The maximum absorption changes observed in those ribonucleic acid experiments (*4*) took place at 260 nm. The activity maximum that I observed at 267 nm does not correlate with the content of nucleic acids in preparations used and it is, therefore, unlikely that this chemically induced activity is due to interactions directly involving the nucleic acid components.

To obtain the biological preparations used in these experiments, the olfactory epithelium covering the turbinates and posterior portion of the nasal septum of the rabbit was gently scraped with a scalpel; the yellow-brown scrapings were suspended in the appropriate medium in a tight-fitting, ground-glass homogenizer. After centrifugation for 30 minutes at 20,000*g*, the clear supernatant fluid was diluted to a final protein concentration between 0.3 and 0.8 mg/ml; the "olfactory preparation" was then ready for use in the experiments.

Further details concerning the preparation of the water-soluble fractions from rabbit olfactory epithelium will be reported elsewhere (*5*). Photomicrographs of olfactory tissue sections before and after the preparations (*5*) demonstrated that the solubles come from the olfactory epithelial tissue distal to the lamina propria which includes the mucus, the olfactory bipolar cells, the sustentacular cells, and the basal cells.

The chemically induced change in absorbance at 267 nm is presented in Fig. 2 for two different concentrations of the same olfactory preparation. As the concentration of the olfactory preparation (measured as milligrams of protein per milliliter) increased, the change in absorbance also increased (Fig. 2a). However, the highest absorptivity (change in absorbance per milligram of protein) was observed at the lower concentration of olfactory preparation (Fig. 2b). Since the stimulant chemical was present at the same concentration in both experiments, competition for the stimulant molecules would favor the higher absorptivity found in the more dilute reaction mixture. It is important to note that the maximum change in absorbance (Fig. 2a) depends on the concentration of the olfactory preparation. The height of the activity plateaus depends on the concentration of the olfactory preparation and not that of the stimulant. The results support the hypothesis that the activity measured by the change in absorbance at 267 nm is due to formation of a complex that involves the stimulant and specific interaction sites in the olfactory prepara-

tion and is not due to enzyme activity. If enzyme activity were being monitored, dilute concentrations of the olfactory preparations would continue to produce measurable activity at a slower rate rather than reach an activity plateau that depends on the concentration of the olfactory preparation.

During the experiments fresh olfactory preparations were sometimes held at room temperature for periods up to 4 hours with very little loss of activity, but all preparations were rapidly inactivated when the temperature was raised to 68°C. This heat sensitivity gives tacit support to the involvement of proteins or lipoproteins. Denaturation at 68°C destroyed activity of the unstimulated preparations and also changed the absorbance characteristics of the preparations that were heated after the activation period. Freezing and thawing had little effect on the activity of the olfactory preparations.

At present the best explanation for the activity measured at 267 nm is that specific ingredients in the olfactory preparations interact with the chemical stimulants to form a complex. The configurational changes associated with formation of this complex would reorient the electron field in the vicinity of the chromophore groups associated with the 267-nm absorbance. Such conformational changes might serve as the trigger to incite the change in mem-

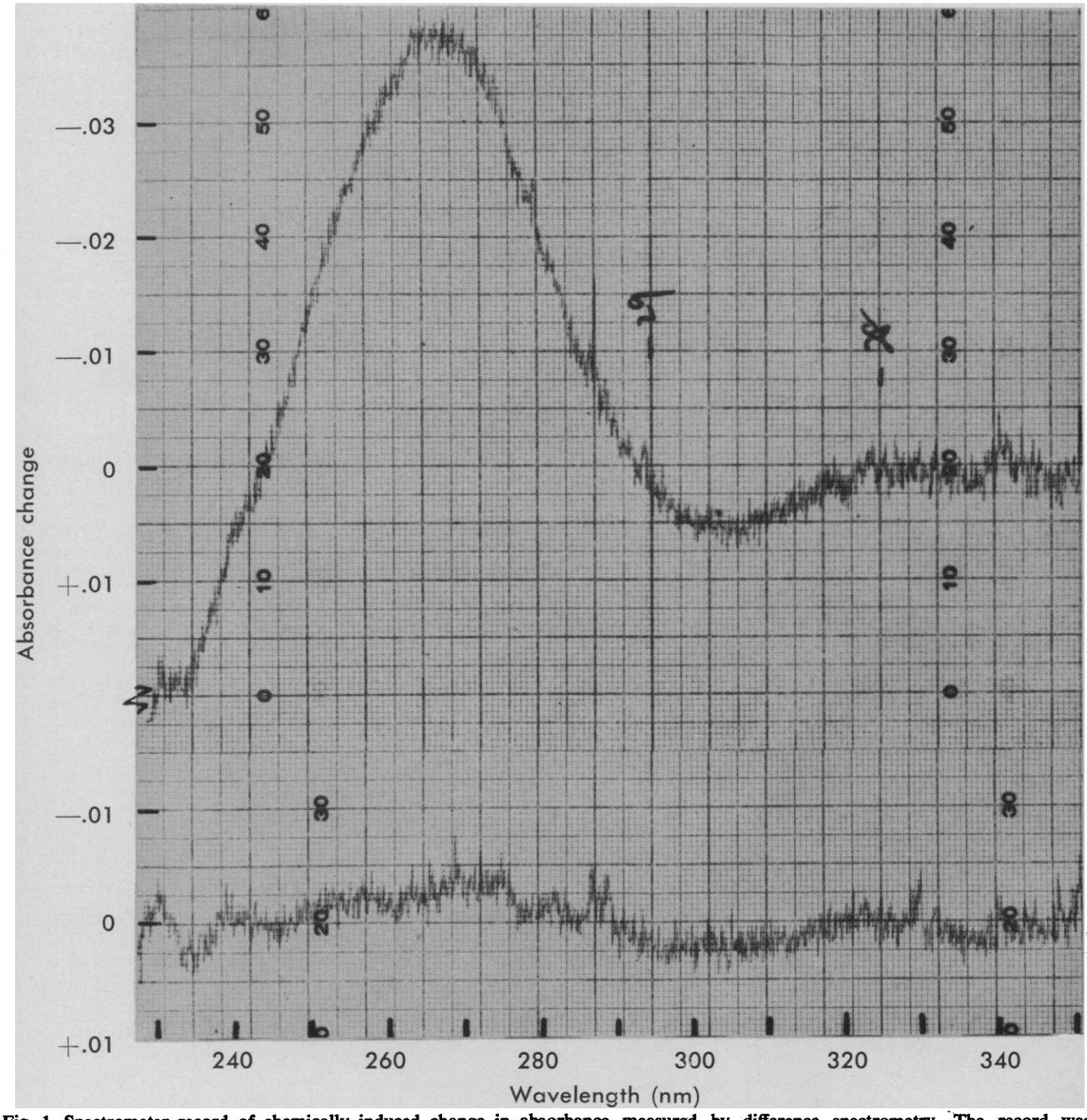

Fig. 1. Spectrometer record of chemically induced change in absorbance measured by difference spectrometry. The record was made with a Cary 14 spectrometer with the use of the 0 to 0.1 absorbance slide wire. The base line spectrum (lower trace) was recorded with the olfactory preparation in both chambers. The upper trace was recorded 30 minutes after 2.0 ml of the olfactory preparation in the reference chamber was mixed with 500 μl of an aqueous solution that was 0.01M for linalool and $<1 \times 10^{-5}M$ for linalyl isobutyrate.

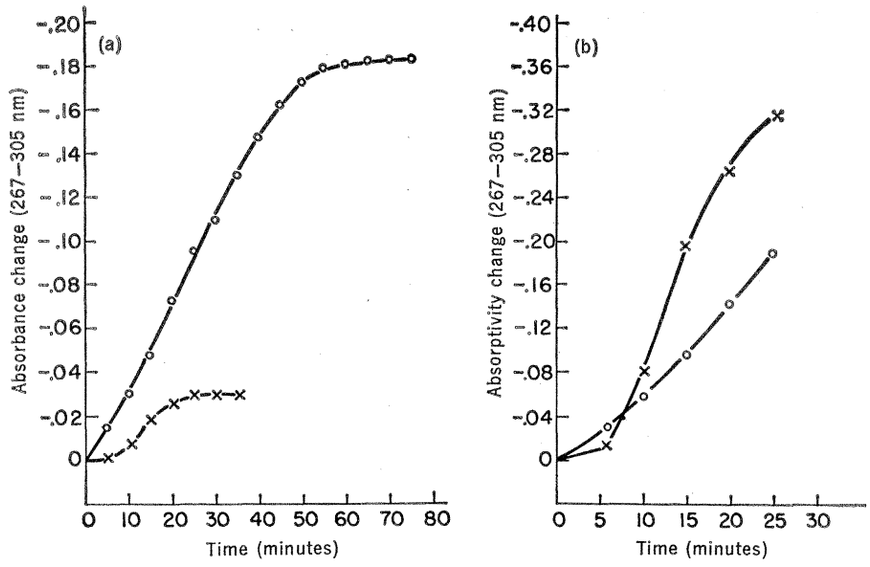

Fig. 2. Effects of the concentration of olfactory preparation on the chemically induced activity measured as change in absorbance (a) and as change in absorptivity (b). The protein concentration of the olfactory preparation was 0.50 mg/ml for the curves labeled O—O. After dilution the protein concentration of the preparation was 0.10 mg/ml (curves labeled X—X). At each concentration the preparation was stimulated with 250 μl of an aqueous solution that was 0.01M for linalool and $<1 \times 10^{-5}M$ for linalyl isobutyrate.

brane permeability needed for chemical sensing by biological systems. The possibility of complex involvement in the olfactory receptor mechanisms has previously been suggested by Dravnieks (*6*), who investigated threshold variations, and more recently by Rosenberg, Misra, and Switzer (*7*).

The activity that was monitored at 267 nm was not unique to the olfactory preparations but was found in varying degrees in preparations from brain and liver, and it is probably present in other biological-chemosensing tissues. The olfactory preparations exhibit the highest activity. In a representative experiment, brain had 41 percent and liver only 7 percent of the specific activity of an olfactory preparation. No chemically induced activity at 267 nm was observed with bovine serum albumin or with preparations of muscle.

The fact that the chemically induced activity was found in preparations of brain and liver does not exclude the possibility that an activity associated with olfactory molecular mechanisms was being monitored. The distinguishing characteristics of the olfactory mechanism might be the structure and location of the bipolar sensing cells rather than molecular mechanisms that are unique to the sensing cells. The olfactory bipolar cells, with their terminal swellings extending into the mucous lining of the nasal cavity and their axons terminating in the olfactory bulb, are ideally suited to facilitate communication between the odor-containing atmosphere and the brain. This unique anatomical arrangement is undoubtedly an important part of the overall mechanisms of olfaction. However, at the molecular level the mechanisms of the olfactory receptors could have much in common with the many other biological chemical sensors.

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References and Notes

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Conductance Changes Associated with Receptor Potentials in Limulus Photoreceptors

Abstract. *The receptor potential in* Limulus *photoreceptors appears to be a consequence not of permeability changes in the cell membrane but of alterations in a light-sensitive constant-current generator.*

The transduction of photic stimuli into neuronal activity in photoreceptor cells results usually in a graded decrease in transmembrane potential, or depolarization, known as the receptor potential (RP). The basic mechanisms underlying its generation are unknown. One hypothesis is that light causes an increase in conductance or permeability of the membrane to one or more ions, particularly sodium (*1, 2*). This conductance increase mechanism (CIM) is essentially the same as that proposed to account for the end-plate potential of the neuromuscular junction (*3*); it has been discussed elsewhere (*1*).

To test the validity of this hypothesis, we have examined a number of electrophysiological properties of photoreceptor cells in the ventral eye of *Limulus polyphemus* (*4*) by means of intracellular recording and electrical stimulation through micropipettes filled with 3M KCl. The organ was deprived of its blood vessel sheath and was mounted in a perfusion chamber, in which the temperature could be varied between 0° and 25°C and the composition of the extracellular fluid could be changed rapidly. All solutions were isosmotic with seawater, buffered at *p*H 7.8, and, where possible, of the same total ionic strength as seawater. Both steady current and up to three independently variable pulses of constant current could be passed through one intracellular microelectrode while changes in potential were monitored through that or a second microelectrode. We determined relations of current and voltage (*I-V* curves) by passing slowly varying currents (about 0.1 cycle/sec) and displaying these and the resulting transmembrane voltages directly on the X and Y axes, respectively, of the oscilloscope. Two independently variable steady or pulsed light beams, passed through heat-absorbing and variable neutral-density filters, were focused onto a single photoreceptor cell.

Three types of electrical activity can be recorded from *Limulus* ventral-eye