The compound-eye tissues containing corneal lens and crystalline cone of the Bombay locust, *Patanga succincta* Johansson, transmitted visible and near ultraviolet light (UV) longer than 280 nm, while the vertebrate (bovine) eye transmitted only visible light of 380 nm or longer wavelengths. From this, the near UV ranging from 280 nm to 380 nm could be considered to have a vital significance to the insect vision and no significance to the vertebrate vision. The photosensitivity and dark reactions of retinyl-thiazolidine-4-carboxylic acid, an insect UV receptor model, indicated that the chemicals such as L-cysteine and hydroxylamine could be considered as the model compounds disturbing the normal function of near UV in the insect eye under dark condition and photosensitive chemicals such as the complexes formed from retinal and L-cysteine or hydroxylamine could be considered as the model compounds giving selective damages to the structure and function of the insect eye upon absorbing near UV. This selectivity was suggested to be utilized as new possible action mechanism of insect-controlling chemicals.

**INTRODUCTION**

The presence of ultraviolet light (UV) sensation in insects has been demonstrated by many workers with behavioral, electrophysiological and biochemical observations. The biochemical studies of the molecular bases of the UV sensation in insect eyes have shown that a UV receptor is the complex formed from a protein component, opsin, and the chromophore, 11-cis-retinal, which is quite similar to the rhodopsin of various animals. The bonding form of these components in the receptor has not been clarified until the recent time. It is assumed similar to Schiff base of rhodopsin, although there is no evidence that Schiff base of retinal has the absorbance peak at the wavelength characteristic to the UV receptor complex.

Recently, Goldsmith proposed an attractive model for the insect UV receptor complex based on the observation made by Peskin and Love. Retinyl-thiazolidine-4-carboxylic acid (RTCA, the absorption maximum at 333 nm), formed from retinal and L-cysteine, mimicked the absorption characteristics of the UV receptor complex. The photosensitivity of this model, however, still remains to be explored as an interesting photochemical process in the insect vision.

One of the aims of this work was to clarify the range of the light energy which could be transmitted through the tissue layer in insect eyes but could be absorbed by the tissues in vertebrate eyes before reaching the photosensitive cells. As an example of insects, the Bombay locust, *Patanga succincta* Johansson, was selected since its compound eye was large enough to be manipulated with certainty. The bovine eye was also used in the experiment as an example of vertebrate eyes.

The other aim of this work was to investigate the photochemical characteristics of RTCA and the effect of hydroxylamine on the system under dark and light conditions.

These conceptions are important in order to develop new selective insect-controlling chemicals based on the peculiar distinction between
the insects and the vertebrates that the insect vision can utilize UV while the vertebrate vision can not and, hence, chemical disturbance of visual UV sensation may be selective to the insects as stated in the previous report.\textsuperscript{7}

**MATERIALS AND METHODS**

1. **Chemicals**
   
   All-trans-retinal was obtained from Eastman Kodak Company, Rochester, New York. L-cysteine monohydrochloride, monohydrate, was obtained from Ajinomoto Co., Inc., Tokyo, Japan. Hydroxylamine hydrochloride and sodium nitroprusside were purchased from Wako Pure Chemical Industries, Ltd., Osaka, Japan. Other chemicals were reagent grade.

2. **Complex Formation**
   
   All-trans-retinal was dissolved in ethanol to make $2.1 \times 10^{-5} \text{ M}$. L-cysteine was dissolved in distilled water to make $2.1 \times 10^{-2} \text{ M}$. Ten ml aliquots of each solution were mixed and, then, 0.5 ml of 2 N sodium hydroxide was added to the mixture. Spectroscopic observations were carried out after standing the mixture at 25°C for more than 15 min. The effect of hydroxylamine was observed by adding 0.04 ml of 200 mM hydroxylamine aqueous solution to 4 ml of the reaction mixture described above.

3. **Sodium Nitroprusside Reaction Test of L-cysteine**
   
   The sodium nitroprusside reaction test of L-cysteine was carried out by the method of Ansong\textsuperscript{19} in the aqueous solution of the crystals that was obtained as a colorless precipitate on the centrifugation of the RTCA solution containing hydroxylamine.

4. **Spectrophotometry**
   
   Fresh bovine eyes were dissected to yield each portion free from other portions of tissues. Liquids were directly added in quarts cells with 1 cm path length and the transmission spectra were obtained against the reference cell filled with distilled water. Solids were spread over the inside surface of a quarts-cell wall so that the uncovered space was in minimum and were wetted with the distilled water. Such a preparation was used for running the transmission spectra against the reference cells whose inner surfaces were moistened with the distilled water.

The compound eyes from alive adults of the Bombay locust, *Patanga succincta* Johansson, were used for the experiment. The tissues containing the corneal lens and crystalline cone were spread closely in contact to each other on the inner surface of a quarts-cell wall. The sample was moistened with quarts water. The transmission spectrum was run against the same reference cell used for the solid samples of bovine eyes.

The transmission spectra of eye tissues were obtained with Hitachi 356 spectrophotometer, which was advantageous for the measurements of the transmittance of diffused samples.

All absorption spectra of retinal solutions were obtained by Toshiba-Beckman DB-GT spectroscopy with quarts cells of 1 cm path length at 25°C.

A fluorescent test lamp (Toshiba FI-3L type, emission-maximum wavelength at 360 nm) was used for the UV illumination of the retinal-complex solutions contained in quarts cells. The illumination experiment was done at 25°C.

**RESULTS**

1. **Transmission Spectra**
   
   Figure 1 shows the transmission spectra of the eye tissues of the bovine and the locusts in the range between 200 nm and 800 nm. The bovine vitreous humor (a) had the highest transmittance. A decrease in transmittance occurred at 320 nm or shorter wavelengths and gave zero at 280 nm. The anterior humor (b) showed 85% transmittance at 800 nm. Its percent transmittance slightly increased as the wavelength decreased to 320 nm (92%), and then rapidly decreased to zero at 250 nm. The retina (c) showed 90% transmittance at 800 nm, which slowly decreased at the wavelengths shorter than 450 nm followed by a rapid decline below 310 nm, reaching zero at 260 nm. The drop in transmittance at 410 nm might be due to the blood vessels attached to the retina. The cornea (d) had 75% transmittance at 800 nm, which decreased at the wavelengths shorter than 450 nm and became zero at 280 nm. The lens (e) showed 85%
transmittance at 800 nm, which decreased slowly at the wavelengths shorter than 550 nm and then abruptly declined to zero at 380 nm. These observations indicated that the lens was the most effective UV filter of the bovine eye tissues.

As shown in Fig. 1 the transmittance spectrum of the insect tissue containing the corneal lens and the crystalline cone showed 75% plateau level in the range between 350 nm and 800 nm followed by a rapid decrease to zero at 280 nm. A comparison of the wavelengths which showed zero transmittance between the bovine lens (e) and the insect corneal lens and crystalline cone (x) revealed that the insect eye transmitted the light ranging from 280 nm to 380 nm whereas the vertebrate lens did not transmit the light of this range.

2. UV Receptor Model
Mixing all-trans-retinal solution with L-cysteine solution resulted in formation of RTCA, whose characteristic absorption maximum occurred at 333 nm as shown in a spectrum of 0 min in Fig. 2. Also shown in Fig. 2 are the spectra of the samples illuminated for the various lengths of time. According to these data, RTCA had a definite photosensitivity with the absorption maximum at 333 nm. When illuminated with UV light (360 nm), the absorbance at 333 nm decreased as a function of the length of time of illumination without any appearance of new characteristic absorbance peak. The acidification of the solution with hydrochloric acid resulted in the disappearance of the absorption maximum at 333 nm without any appearance of new absorbance peak at the wavelengths longer than 300 nm.

The effect of hydroxylamine on RTCA was examined in the presence or absence of hydroxylamine under 40-min UV illumination or dark condition. The results are shown in Fig. 3. The spectrum 3 clearly showed that the addition of hydroxylamine caused the red shift of the absorbance peak from 333 nm to 360 nm, indicating the conversion of RTCA into Schiff base of retinal under the dark condition. The photobleaching of retinylidene hydroxylamine was apparent as seen in the spectrum 3'.
in which there was no shift of the major absorption peak. The photobleached RTCA (2) was also found to be able to form retinylidene hydroxylamine when added with hydroxylamine under the dark condition (from the spectrum 2 to 2'). An exposure to UV in the presence of hydroxylamine resulted in bleaching at 333 nm and red shift to 360 nm as shown in the spectrum 4. The greatest bleaching effect was obtained in the presence of hydroxylamine under UV.

Whenever hydroxylamine was added to RTCA, formation of white precipitate was clearly observed. The negative result of the sodium nitroprusside reaction test indicated that the precipitate was not L-cysteine but presumably L-cystine.

**DISCUSSION**

1. **UV Transmission in the Insect Eye**

In the bovine eye, liquids were more transparent than solids. All components cut the light below 280 nm. The substances responsible for the absorption of the light of 280 nm or shorter wave lengths may be proteinous components in these tissues. The substances responsible for the absorption of the light of longer wavelengths upto 380 nm may be other small molecular weight compounds specific to these tissues. In the retina hemoglobin significantly modifies the transmission spectrum. It is quite interesting that the absorption peak of hemoglobin can be masked by that of the lens. Unlike the insect retina, the vertebrate retina is in inversed state with respect to the direction of the incident light and, hence, the light interacts with hemoglobin before interacting with rhodopsin in rods and cones. The absorption of near UV below 380 nm by the lens may be considered as an adaptive measure for the removal of this disadvantageous absorption due to hemoglobin in the vertebrate eye.

It is evident from the result of this experiment that near UV, covering from 280 nm to 380 nm, may be considered to reach the receptor complex in the insect eye and not to reach the retina in the vertebrate eye. This point is significant since the near UV can affect only the insect eye but not the vertebrate eye and the presence of the photosensitive compounds absorbing light energy at this region may have decisive influences on the function of the insect eye.

Near UV, longer than 300 nm, reaches to the surface of the earth from the sun. Hence, it must be kept in mind that near UV can affect only the insect eye but not the vertebrate eye and the normal visual function may depend on the action of the near UV required by a given insect in the natural conditions. Disturbing this normal function by photosensitive compounds may initiate harmful reactions to disrupt the structure and function of the insect eye. This type of photosensitive compounds may be effectively utilized as insect-controlling chemicals since the solar energy is practically limitless.
2. Photosensitivity of an Insect UV Receptor Model

Peskin and Love showed that RTCA formation caused the blue shift of retinal absorption maximum to 333 nm in 50% ethanol water system. In the present work, it was observed that the acidification of the 50% ethanol water system resulted in simple bleaching without causing the red shift of the absorption peak in RTCA solutions as reported by Peskin and Love. The reaction was irreversible even in this type of the dark reaction in the retinal component.

The addition of hydroxylamine to stabilized RTCA caused the red shift of the absorption maximum from 333 nm to 360 nm. This may be due to retinylidene hydroxylamine formed by the dark reaction of hydroxylamine after the dissociation of RTCA. The observation of L-cystine formation at the same dark reaction indicated the irreversibility of the reaction in the L-cysteine component.

UV illumination of RTCA resulted in the simple disappearance of the absorption maximum without L-cystine formation. The light at the presence of hydroxylamine caused the satulation of retinal as well as L-cystine formation.

The UV illumination on retinylidene hydroxylamine also resulted in a decrease of absorbance at 360 nm without appearance of retinal peak. This may be due to the saturation of retinal. Figure 4 summarizes the dark and light reactions observed in the present work.

RTCA, due to its high photosensitivity, seems to be a good working model for the insect UV receptor. At the same time, it is very likely that one of the roles of opsin is to protect retinal from various photochemical reactions and, consequently, to protect animals from the photoproducts generated in the interaction of light with retinal.

3. Selective Inhibition of Insect UV Vision

Now, let us examine a possibility of utilizing L-cysteine as visual inhibitor of insect eye. The effect of L-cysteine on insect vision should be considered in two steps, namely, the dark reaction with retinal to form RTCA and the photoreactions of RTCA.

The physiological significance of RTCA formation in dark reaction is the competitive removal of retinal from its visual cycle. Consequently, it causes the insect eye more susceptible to near UV by the blue shift of the absorbance peak.

The effects of the photoreaction of RTCA may be divided into three categories, namely, 1) light absorption, 2) saturation of retinal, and 3) generation of photoelectric membrane potentials:

1) Simple absorption of light by RTCA in the insect eye may decrease the intensity of near UV at the vicinity of the receptor cells so that the normal absorption processes and color matching of the incident light by UV receptor complex and pigment granules may be distorted.

2) The saturation of retinal by the photoreaction of RTCA may cause a definite damage to UV receptors and visible light receptors since it means the photodegradation of retinal which results in the removal of retinal from its visual cycle quite similar to the dark reaction in RTCA formation.

3) If RTCA can exist in the membranous structure, the absorption of near UV by this pigment may generate photoelectric membrane potentials and, hence, modify receptor mem-
brane potentials since the photoelectric potentials have been demonstrated to be generated in membranous systems using retinal bilayer lipid membrane.\(^{10-14}\)

Discussed above, the dark reaction of L-cysteine and retinal and the photoreactions of RTCA may be considered to give selective damages to the insect eye comparing to the vertebrate eye (See Fig. 5).

The results obtained by the interactions between hydroxylamine and retinal indicate that Schiff base forming agents\(^{14,15}\) may also be used as visual inhibitors. The dark reaction may result in the competitive inhibition of formation of a retinal-opsin linkage. The light absorption by the retinylidene compounds formed may affect the spectral acuity of the UV receptors and visible light receptors, the saturation of retinal will disrupt the visual cycle, and photoelectric effects will disturb the normal function of receptor cell membranes. Due to the selective UV transmission, a greater photochemical damage will occur in the insect eye than in the vertebrate eye.

Misato and his coworkers have been engaged in developing the amino acid related fungicides which may not cause environmental pollution.\(^{16}\) Since amino acids include thiazolidine forming agents and Schiff base forming agents, the amino acid related insecticides which may not bring out environmental pollution would now become feasible in principle. Indeed, in the author’s laboratory, the screening of amino acids working along the hypothesis proposed in this report resulted in the finding of the compounds showing unambiguous damages to the insect vision.\(^{17}\)

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Fig. 5 The selective action mechanism of L-cysteine on the UV sensation in the insect eye.

The dark reaction with retinal disrupts the visual cycle and sets up a complete UV selectivity. The action of the insect-selective sunlight on RTCA results in the various photochemical effects as discussed in the text.

a: Sunlight on the surface of the earth, b: Light transmission in vertebrate eye, c: Light transmission in insect eye, d: Insect-selective sunlight, e: Half absorption band width of retinal, f: Half absorption band width of RTCA.

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