全トランスーレチナール脂質二重膜における L-システインの光化学反応(農薬生産用農産食料製造)(農芸化学科)

Pesticide-producing Agricultural Food Processing. The photochemical reactions of L-cysteine on all-*trans*-retinal bilayer lipid membranes*

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I INTRODUCTION

Investigations on L-cysteine, a potential food-originated pesticide, in a molecular level, using bulk solutions of all-*trans*-retinal, i.e. a target compound of the action of L-cysteine, resulted in a proposition of the mechanisms of selective inhibition of insect vision[3]. In the present work, the mode of the photochemical reactions between all-*trans*-retinal and L-cysteine was investigated using a model membrane system, the bilayer lipid membrane (BLM) containing all-*trans*-retinal[4,5], since the all-*trans*-retinal generated by the action of light in receptor membrane would interact with L-cysteine on the membrane. Especially, the present investigation made a comparative study on this interaction in a bulk solution system and BLM system at the various pH of the aqueous phases. The mechanisms of the action of L-cysteine on the photoresponses of all-*trans*-retinal BLM and the applicability of the membrane system to screening the possible food-originated insecticides acting on the early processes of insect vision were also discussed.

II MATERIALS AND METHODS

1 Chemicals

L-cysteine was obtained from Ajinomoto Co., Ltd., Tokyo. All-*trans*-retinal was purchased from Eastman Kodak Company, Rochester, New York. Potassium ferricyanide and ferric chloride were obtained from Wako Pure Chemical Industries, Ltd., Osaka. Other chemicals were in reagent grade as obtained from manufacturers.

2 Bulk solution system

Potassium ferricyanide was dissolved into buffers to make up 5 x 10^{-4} M aqueous solutions. All-*trans*-retinal was dissolved into ethanol and then diluted with buffer solutions to make 50%-ethanol aqueous solution of 7.7 x 10^{-6} M of all-*trans*-retinal. The various buffers were used for adjustment of

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pH of the solution, i.e., 0.2 M sodium acetate buffer (pH 4.2-5.5), 0.2 M sodium phosphate buffer (pH 6-7), and 0.2 M boric acid buffer (pH 8-9). The above solutions (3 ml) of potassium ferricyanide or all-*trans*-retinal were contained in test tubes (16.5 mm x 160 mm) and were illuminated by the light condensed through the lens and cupric sulfate heat-filter placed infront of a 1000-W tungsten lamp for 10 min in the case of potassium ferricyanide or for 5 min in the case of all-*trans*-retinal. The absorption spectra were obtained at 25°C after 15-min interaction for the un-illuminated samples or immediately after the illumination for the illuminated samples using a Toshiba-Beckman DB-GT spectroscopy with a quarts cell of 1.00-cm path length. The equilibrium constant of formation of all-*trans*-retinyl thiazolidine-4-carboxylic acid (RTCA)[6] and the extinction coefficient at 333 nm of RTCA absorption peak under the experimental conditions of the present work were evaluated by the method of Ketelaar and his co-workers[1].

3 Membrane formation and photopotential measurement

The preparation of all-*trans*-retinal lipid solution and the techniques of membrane formation as well as the instrumentation including membrane-forming chamber, potential measurement and illumination were reported elswhere[4,5].

All-*trans*-retinal BLM was formed in pH 5, 0.2 M sodium acetate buffer solution. When L-cysteine dissolved into the same buffer solution was added into the inner chamber, ferric chloride or potassium ferricyanide dissolved into the same buffer solution had been added into the outer chamber as electron acceptor. The temperature of the membrane-forming chamber was maintained at 25°C by circurating thermostated water through the bottom chamber of the membrane-forming cell. The pH of the aqueous phases was adjusted in a similar manner as stated in the section of the bulk solution system. The equilibrium constant of RTCA formation at the interface of a BLM and the maximum inhibition at an extremely large concentration of L-cysteine were evaluated by the method described elswhere[2,4].

III RESULTS AND DISCUSSION

1 The effect of pH on the photoresponses

The photoresponses of all-*trans*-retinal BLM were remarkably dependent on pH of the aqueous phases of a BLM as shown in Fig. 1. There was the maximum of the photoresponses at pH 4.6 and reached to a lower stable value at pH 6.0. Since ferric chloride and potassium ferricyanide, electron acceptors, showed a satisfactory agreement at pH 5.0-5.5, the pH dependence of the BLM photoresponses did not seem to be due to the nature of the electron acceptors. This point was also seen in the independency of the position of absorption maxima and the absorbances at the wavelengths as shown in Fig. 2. And, furtherm ore, the fact that the absorbance at these wavelengths of potassium ferricyanide were not affected by illumination regardless pH values indicated that the above reasoning was acceptable.

The absorbance (at 387 nm) of all-*trans*-retinal and its bleaching (%) were also independent of pH as shown in Fig. 3. From the pH independency of the electron acceptors and all-*trans*-retinal, the pH

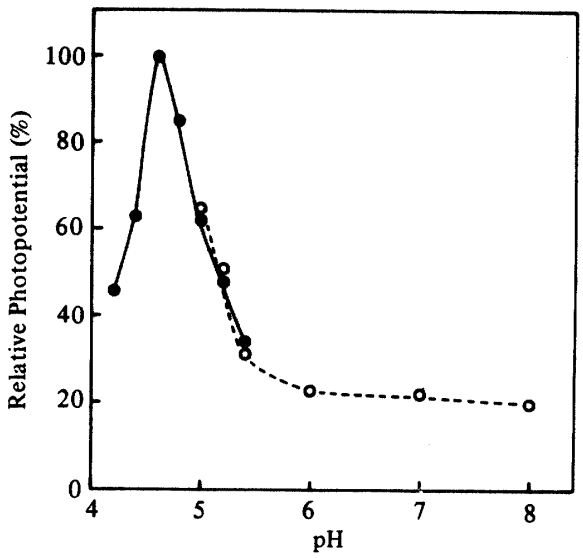

Fig. 1. The effect of pH of the aqueous phase on the photopotentials of an all-*trans*-retinal
●——●; Ferric chloride as electron acceptor,
o----o; Potassium ferricyanide as electron acceptor

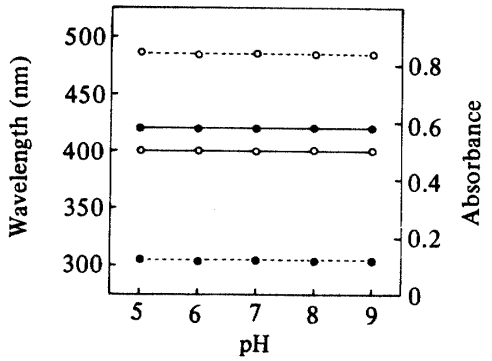

Fig. 2. The effect of pH on the absorption maxima and absorbance of 5 x 10^{-3}M potassium ferricyanide aqueous solution
●——●: Wavelength of the visible absorption maximum,
●-----●: Wavelength of the near-UV absorption maximum,
o——o: Absorbance at the visible absorption tion maximum,
o-----o: Absorbance at the near-UV absorption maximum

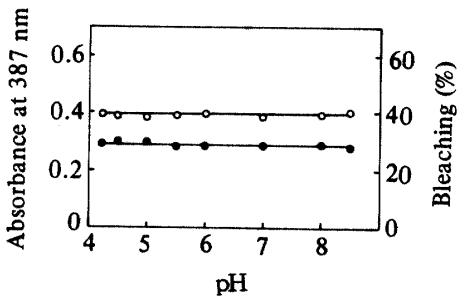

Fig. 3. The effect of pH on the absorbance at 387 nm of 9.2 x 10^{-6} M all-*trans*-retinal in 50%-ethanol water
o——o : Absobance at 387 nm,
●——● : Percentage bleaching under 5-min illumination with white light

dependency of the photoresponse may be considered to come from the properties of the BLM itself. One of the possibilities is that the charge carriers involved in generation of the photoresponses are positive spiecies such as holes as proposed elswhere[4,5], since acidic environment may stabilize the existence of these charge carriers.

2 The effect of pH on the action of L-cysteine

After formation of all-*trans*-retinal BLM in 0.2 M acetate buffer, with various pH values, as aqueous phases, ferric chloride was added into the outer phase to make up a concentration of 1 x 10^{-3} M solution. In order to determine a stable photopotential for 1-sec illumination under the influence of ferric chloride, the photopotentials were repetitively observed as a function of reaction time at this concentration. After obtaining a stable potential, an aliquat of L-cysteine solution was added into the inside phase to make up a concentration of 1 x 10^{-2} M under stirring of the aqueous phase. Again, the photopotentials were repetitively evaluated as a function of time. The time courses of the above measurements (at pH 4.8 and 5.0) are shown in Figs. 4 and 5, respectively. A

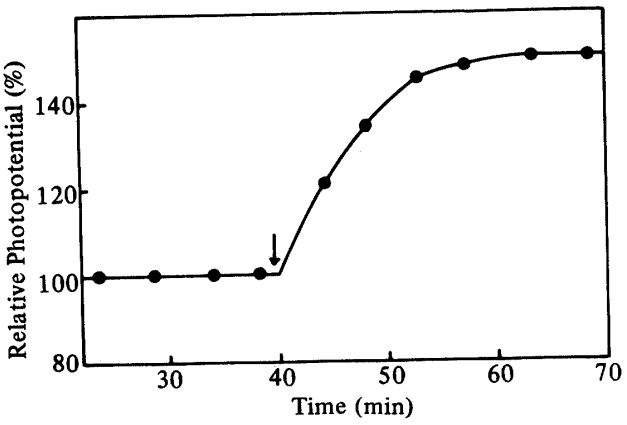

Fig. 4. The time course of the enhansive effect of 1 x 10^{-2}M L-cysteine on the photopotentials of an all-*trans*-retinal BLM at pH 4.8
The downward arrow indicates the time of adding L-cysteine into the aqueous phase.

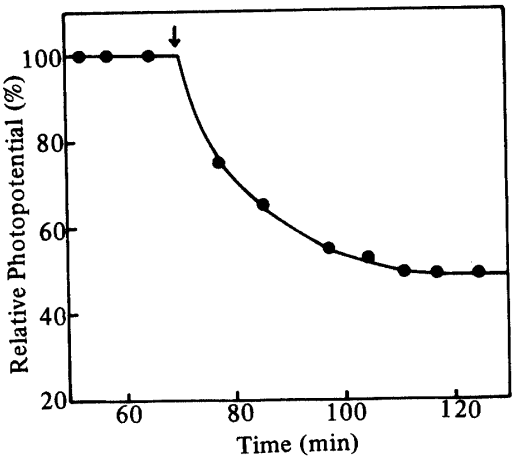

Fig. 5. The time course of the reductive effect of 1x 10^{-2}M L-cysteine on the photopotentials of an all-*trans*-retinal BLM at pH 5.0
The downward arrow indicates the time of adding L-cysteine into the aqueous phase.

slite change in pH values from 4.8 to 5.0 modified the photoresponses in diminishing direction. Indeed, the investigation of the pH dependence of the effect of L-cysteine over a range of pH 4.2-5.4 showed the presence of the maximum of enhancive effect at pH 4.8 and a reductive effect at the pH values higher than 4.9 as shown in Fig. 6.

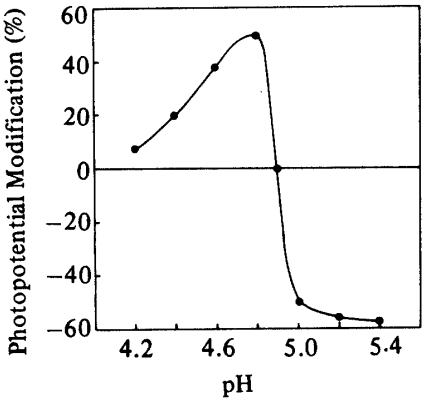

Fig. 6. The pH dependence of the effect of 1 x 10^{-2}M L-cysteine on the photopotentials of an all-*trans*-retinal BLM
The aqueous phase was 0.2 M sodium acetate buffer. An electron acceptor was 1 x 10^{-3} M ferric chloride located in the outer aqueous phase opposite to the L-cysteine contained in the inner aqueous phase.

An enhancive effect of L-cysteine may be considered as a result of electron donation by L-cysteine into a BLM since L-cysteine is a reasonably functionable reducing agent. The reductive effect observed at higher pH values may be related to formation of RTCA since the RTCA formation is reported to be pH dependent, i.e. the rate of formation is high at elevated pH values[6].

3 RTCA formation

In order to understand a mode of interaction of L-cysteine with all-*trans*-retinal in the BLM at higher values of pH, the formation of RTCA from all-*trans*-retinal and L-cysteine was examined in the 0.2 M acetate buffer (pH 5) containing 50% ethanol. Since RTCA formation was reported to occur readily at pH 5[6] and its characteristic absorbance at 333 nm reached to an equilibrium point at about 60 min, the association constant of the RTCA formation was evaluated at this reaction time by the method of Ketelaar and his co-workers[1] as shown in Fig. 7. From the linear relationship shown in Fig. 7, the association constant was obtained as 106 *l*/M and extinction coefficient of

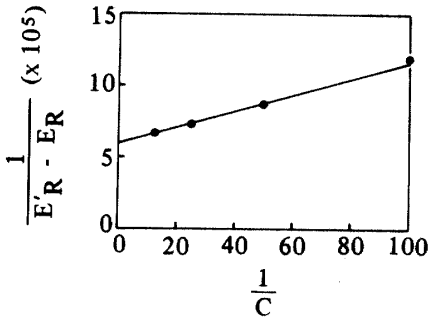

Fig. 7. The double receiprocal plot of the concentration of L-cysteine and the difference between the apparent molar extinction coefficient (E'_R) and the molar extinction coefficient (E_R) of all-*trans*-retinal at 333 nm for the method of Ketelaar and his co-workers

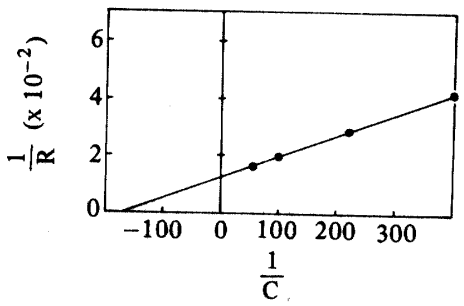

Fig. 8. The double reciprocal plot of the concentration of L-cysteine and the reductive effect (%) on the photopotentials of an all-*trans*-retinal BLM at pH 5.0

RTCA band (333 nm) as 32800 *l*/Mcm. Assuming that RTCA formation causes the reductive effect on the photopotentials of all-*trans*-retinal BLM, the association constant of 120 *l*/M was evaluated from the linear relationship obtained using the extent of reductive effect (%) on the photopotentials as the values of responses as shown in Fig. 8. Although the latter association constant is slightly greater than the former constant evaluated, a hypothesis of regarding RTCA formation as a major cause of a decrease in the photopotentials cannot be rejected. That is, a decrease in the photopotential due to the RTCA formation at the membrane interface may be attributed to the shift of absorption peak to the value being lower than the limiting wavelength (360 nm) of the light effective for the excitation of the present membrane system. At the same time, a larger value of the association constant for the membrane system relative to that for the solution system may be considered in the following manner. That is, the hydrolytic dissociation of the bond

of the RTCA, which may occur in the bulk solution phase, may be reduced by the immersion of the portion of the thiazolidine ring into the hydrophobic region of the membrane as shown schematically in Fig. 9. It is also indicated in Fig. 9 that the molecular orientation at the hydrophobic region is altered by the RTCA formation. That is, conjugated double bonds in all-*trans*-retinal can reach to

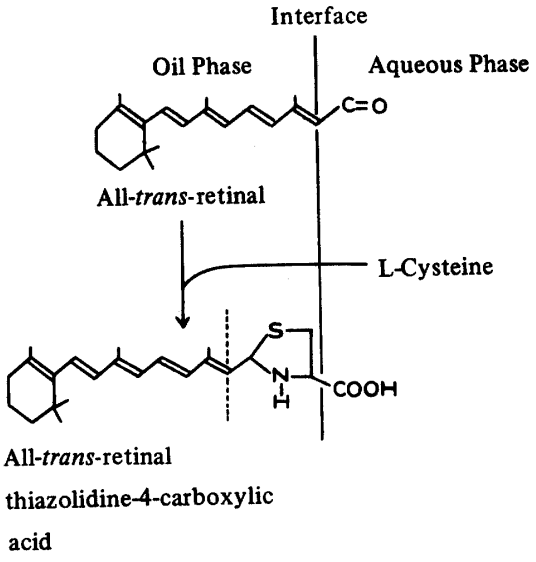

Fig. 9. A schematic diagram showing the pigment orientation at the interface of BLM before and after a reaction between all-*trans*-retinal and L-cysteine

the region close to the aqueous phase of BLM while that of RTCA is located in significantly distant position. Such a modification of the molecular orientation of the pigment may also results in a decrease in the photo-electric trunsduction of visible light by the present BLM in addition to the spectral band shift mentioned above.

4 Applicability to screening

A desirable pH value for screening RTCA formation at membrane level may be considered as physiological pH value or higher since greater formation occured at elevated pH values[6]. The pH dependence of the photopotentials of all-*trans*-retinal BLM had the maximum response at pH 4.6 and lower responses at pH 7 (Fig. 1). From this point, pH 5 was considered as the proper pH value for the purpose of screening the L-cysteine-like inhibitors. Ferric chloride or potassium ferricyanide would be used as an electron acceptor. The maximum effect estimated from the linear relationship shown in Fig. 8 at an extremely large concentration of L-cysteine was 87%, indicating that the photopotentials were sensitive enough to detect the reaction of L-cysteine and, hence, the possible inhibitors forming RTCA.

Finally, it became quite evident that this membrane system would be utilized for screening the food-originated inhibitors acting in a way similar to L-cysteine as much as understanding the mechanism of the action of L-cysteine on visual receptor cells.

IV SUMMARY

The light-induced potentials of an all-*trans*-retinal bilayer lipid membrane were influenced by the presence of L-cysteine in the aqueous phase, 0.2 M sodium acetate buffer, having an electron acceptor, ferric chloride, in the opposite aqueous phase separated by the membrane. In a pH range up to 4.9, an enhancive effect was observed with the maximum at 4.8, whereas in a pH range beyond 4.9 a reductive effect was observed. The former enhancive effect was explained as a redox-related effect of L-cysteine on the major charge carrier, hole, of the photopotentials observed. The latter reductive effect was explained as a spectrophotometric effect of formation of all-*trans*-retinyl thiazolidine-4-carboxylic acid at the membrane interface. This membrane system was presented to be used for screening the vision inhibitors acting in a way similar to L-cysteine as well as understanding the mechanisms of the action of these potential food-originated pesticides on the insect vision.

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農薬生産用農産食料製造－全トランス－

レチナール脂質二重膜における
L－システインの光化学反応

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要　　約

膜で隔てた外室に電子受容体として塩化第二鉄を含む酢酸緩衝液（0.2M）中での全トランス－レチナール脂質二重膜は，内室へのL－システインの添加により，光電位の変化を示した。pH値が4.9以下では増強効果が認められ，4.8において最高となり，一方，4.9より高い領域では減少効果が認められた。前者の増強効果は，光電位発生の主荷電体，ホール，に対するL－システインの酸化還元作用として説明された。後者の減少効果は，界面における全トランス－レチニルチアゾリジン－4－カルボン酸形成の分光学的影響として説明された。この膜系は，昆虫の視覚に作用する食料起源の農薬の作用機構の理解と共に，L－システインと同様に作用する視覚阻害剤のスクリーニングにも利用できることが示された。

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