全トランスーレチナール脂質二重膜における反応の定量的評価法(農薬生産用農産食料製造)(農芸化学科)

Pesticide-producing Agricultural Food Processing. A method of quantitative evaluation of reactions on all-*trans*-retinal bilayer lipid membranes*

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I INTRODUCTION

The purpose of the present work was to explore the possibility of using an all-*trans*-retinal bilayer lipid membrane (BLM)[8,9,13] for studying the mechanisms of the action of food-originated insecticides on energy-transfer processes in insect photoreceptors and screening potential insecticides at a membrane level. Tetrachloro-1,4-benzoquinone (chloranil; Spergon fungicide) was selected as the model agent being capable of forming a charge-transfer complex (CTC)[1] with all-*trans*-retinal in a bulk solution and at the BLM interface. The CTC formation at the two phases was quantitatively evaluated and reported for future utilization of the BLM system for studies on pesticide-membrane interactions.

II MATERIALS AND METHODS

1 Chemicals

Chloranil was obtained from Tokyo Kasei Kogyo Co., Ltd., Tokyo. All-*trans*-retinal was purchased from Eastman Kodak Company, Rochester, New York. Other chemicals were of reagent grade as obtained from manufacturers.

2 The measurement of the photopotentials of all-*trans*-retinal BLM

The preparation of all-*trans*-retinal lipid solution and techniques of membrane formation as well as instrumentation including membrane-forming chamber, electric measurement, and light illumination were similar to those reported elswhere[8,9]. A slide projector with a 1000-W tungsten lamp was used as a light source. A Keithley electrometer Model 610C with a Yokogawa recorder Model 3046 was utilized in the present work for the measurement of photopotentials.

3 The determination of the effect of chloranil on photopotentials

Chloranil dissolved into sodium acetate buffer (pH 5, 0.2 M) was added into the inner aqueous phase while ferric chloride dissolved into the same sodium acetate buffer was added into the outer aqueous

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phase to make up a concentration of 1 x 10^{-3} M as an electron acceptor. The temperature of the membrane-forming chamber was maintained at 25°C by circulating thermostated water with Komatsu-Yamato Coolnics circulator Model CTE-120 through the bottom chamber of the membrane-forming cell.

4 Spectrophotometry

To form a CTC of all-*trans*-retinal with chloranil, retinal ethanol solution (2 x 10^{-4} M) and chloranil ethanol solution (2 x 10^{-4} M) were mixed in various molar ratios and, then, added to sodium acetate buffer (pH 5, 0.2 M) to make 10%-ethanol aqueous solutions. In a similar manner, solutions with various percentages of ethanol were used for the experiment determining a proper ethanol content. The absorption spectra were obtained at 25°C with a Toshiba-Beckman DB-GT spectrophotometer using a silica cell of 1.00-cm path length. The composition, association constant, and extinction coefficient of the CTC were obtained by the plot of a continuous variation method[14].

III RESULTS

1 The effect of chloranil on photopotentials

An addition of chloranil into the inner aqueous phase increased the photopotentials observed by 1-sec illumination of the all-*trans*-retinal BLM having 1 x 10^{-3} M ferric chloride in the outer aqueous phase. This enhancement effect was significantly multiplied as the concentration of chloranil was increased. The dark membrane potentials, however, were essentially unmodified.

In order to analyze the enhancement effect of chloranil on the photopotentials induced, an equation was developed.

Let us assume that the magnitude of an enhancement effect (E) is proportional to the concentration of the complex (RC) formed by the interaction of all-*trans*-retinal (R) and chloranil (C) at the BLM surface. Then, the following equation can be readily obtained as described for the general case of drug-receptor interactions by Goldsteine et al[4]. That is, for:

$$R + C \underset{k_2}{\overset{k_1}{\rightleftharpoons}} RC, \qquad 1$$

$$E = k_3 (RC), \text{ and} \qquad 2$$

$$K = k_1/k_2 = (RC) / (R) (C), \qquad 3$$

then,

$$1/E = 1/KE_m (C) + 1/E_m, \qquad 4$$

where K is the association constant of the complex and E_m is the magnitude of the maximum effect when C becomes extremely large. When reciprocal response magnitudes (1/E) are plotted against reciprocal concentrations of chloranil (1/C) a straight line with slope $1/KE_m$ and intercept $1/E_m$ is obtained.

As shown in Fig. 1, Eq. 4 well fits the data obtained in the present work when E values were

expressed as the percentages of the photopotentials observed at various concentrations of chloranil to

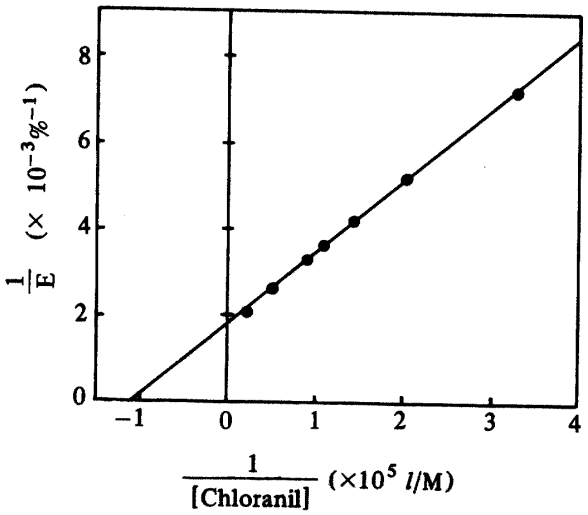

Fig. 1. The double reciprocal plot of the effect of chloranil on the photopotentials of all-*trans*-retinal BLM
An effect (E) was expressed as a percentage of the photopotencial observed for 1-sec illumination at about 30 min after the addition of a given concentration of chloranil to that of all-*trans*-retinal BLM at the absence of chloranil. An E value was evaluated for each BLM used for given concentration of chloranil.

the photopotentials obtained at the absence of chloranil in the inner aqueous phase. The values of K = 1.1 x 10^5 *l*/M and E_m = 570% were obtained from Eq. 4 together with the straight line shown in Fig. 1.

2 The formation of a CTC of all-*trans*-retinal with chloranil

The formation of a CTC of all-*trans*-retinal with chloranil was studied with ethanol aqueous solution. All-*trans*-retinal and chloranil did not form a CTC in ethanol. When sodium acetate buffer was added to the above ethanol solution, a new broad absorption band at a longer wavelength, 450 nm, was observed as shown in Fig. 2. Since the characteristic absorption bands at 426 nm and 455

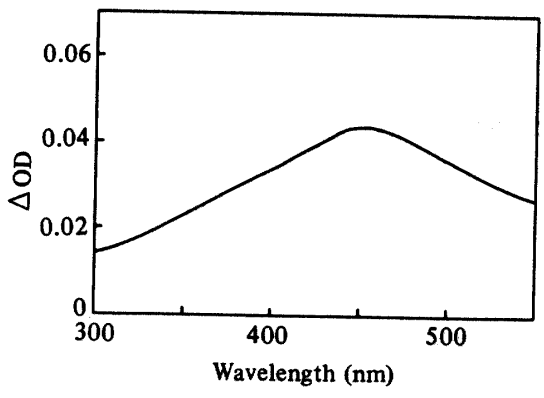

Fig. 2. The difference absorption spectrum of the 10%-ethanol acetate buffer (pH 5, 0.2 M) containing all-*trans*-retinal (1.0 x 10^{-5} M) and chloranil (1.0 x 10^{-5} M) after interaction for 75 min against the individual components hald in separate cells

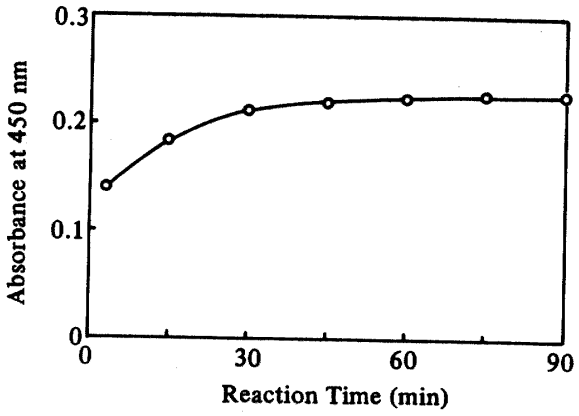

Fig. 3. The time dependence of the absorbance at 450nm of the 10%-ethanol acetate buffer (pH 5, 0.2 M) containing all-*trans*-retinal (1.0 x 10^{-5} M) and chloranil (1.0 x 10^{-5} M)

nm of chloranil anion in water[1] and that at 594 nm of all-*trans*-retinylic cation in ethanol[2] were not observed in the above spectrum, a broad absorption band at 450 nm would be attributed to a CTC of an unionized molecular complex. The extent of CTC formation was dependent on the ethanol content of the solution such that a CTC band at 450 nm increased with a decrease in ethanol content and stayed constant at the ethanol content of 10% or lower percentages. From these observations, 10%-ethanol aqueous solution was used for the subsequent work presented in this report.

The time dependence of the major absorption band of a CTC at 450 nm is shown in Fig. 3. Formation of the CTC was almost constant after 60 min. From this, a reaction for 75 min was considered to be sufficient for completing the formation of the CTC in 10%-ethanol aqueous solutions.

The plot of the continuous variation method for a CTC of all-*trans*-retinal and chloranil is shown in Fig. 4. The stoichiometry of CTC formation showed mole-for-mole interaction between a donor and an

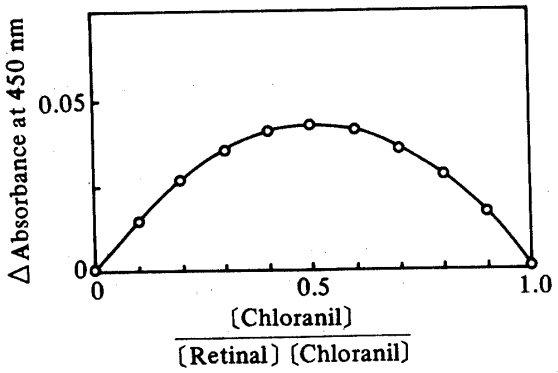

Fig. 4. The plot of the continuous variation method for the complex of chloranil with all-*trans*-retinal
Difference absorbance was measured between the complex solution and the individual components contained in separate cells. The total concentration of all-*trans*-retinal and chloranil was 2 x 10^{-5} M. The reaction time was 75 min.

acceptor. The association constant of the CTC was 2.6 x 10^5 *l*/M and the extinction coefficient of a CTC-band at 450 nm was 7450 *l*/Mcm.

IV DISCUSSION

It is known that all-*trans*-retinol and all-*trans*-retinoic acid can form a CTC with chloranil[10]. From the spectroscopic data presented in this work formation of CTC of all-*trans*-retinal with chloranil is also evident.

The K value obtained from bulk solution was 2.6 x 10^5 *l*/M while that of membrane system was 1.1 x 10^5 *l*/M. The agreement between these values is indicative of formation of a CTC in the BLM system. Chloranil is well know as a π-acceptor. All-*trans*-retinal, on the other hands can be expected to function as a donor and acceptor of π-electron from properties of carotenoids[12]. According to unpublished data obtained in author's laboratory, all-*trans*-retinal also seems to function as a donor and an acceptor in molecular association. Because of these points, it is more likely that π-π interaction is the major form in the complex of all-*trans*-retinal with chloranil in a quite similar way to β-carotene association[11]. A lower K value in the membrane system than that in the bulk

solution system may also indicate the formation of π-π complex since the penetration by chloranil into the interface of a BLM can be one of rate-limiting factors in the CTC formation in the BLM system and such a limiting factor is essentially negligible in the bulk solution system.

Using Briegleb equation[3]:

$$h\nu_{CT} = I - C_1 + C_2 / (I - C_1), \qquad 5$$

and constants, C_1 = 5.70 eV and C_2 = 0.44 eV, for π-CT of chloranil and energy of CT-band, $h\nu_{CT}$ = 2.76 eV, of the present CTC, the ionization potential of all-*trans*-retinal, I, was obtained as 8.47 eV. This value falls within the range of the I values of aromatic donors of π-electron to chloranil[3].

In order to explain an enhancement effect of chloranil on BLM photopotentials, the molecular organization of a CTC at BLM surface, or interface, needs to be considered. Since all-*trans*-retinal molecules are located within a BLM and its CTC is not formed in ethanol, at the absence of an aqueous solution, chloranil is expected to be adsorbed onto the BLM interface facing the inner aqueous phase as schematically shown in Fig. 5. This molecular organization at the BLM interface is compatible to a requirement of hydrophylic environment for formation of the CTC. Because of this

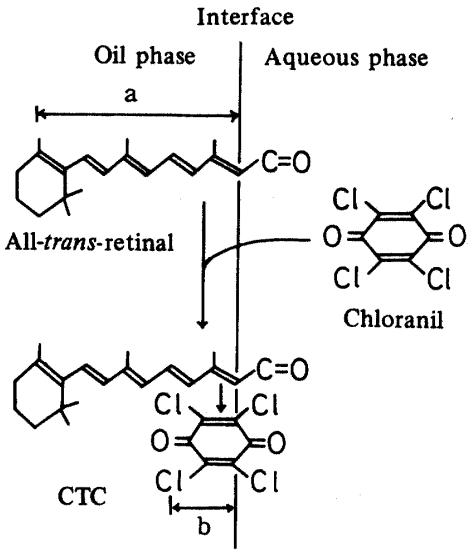

Fig. 5. A schematic diagram showing the effect of chloranil on the distribution of the π-electron of all-*trans*-retinal in a BLM due to formation of a CTC
a: Delocalization over uncomplexed all-*trans*-retinal, b: Induced localization over complexed chloranil due to CTC formation

type of molecular organization at the BLM interface, inducing the localization of electrons at the region close to the inner interface of the BLM, the holes[8,9] generated from exciton by ferric chloride that exists at the outer interface of the BLM may be attracted toward the inner interface and hence may increase the diffusion of the holes, resulting in an enhancement in the light-induced potentials.

In this work, a use of all-*trans*-retinal BLM to investigate the action mechanisms of chloranil revealed the unique mode of action of this energy-transfer inhibitor. It is also worthy to mention that a use of this model membrane system may contribute toward screening and elucidating the action mechanisms of potential food-originated pesticides such as energy-transfer inhibitors in insect vision[7].

V SUMMARY

In order to develop the methods of quantitative evaluation of the action of food-originated pesticides on the bilayer lipid membrane, a charge-transfer complex (CTC) of tetrachloro-1,4-benzoquinone (chloranil) and all-*trans*-retinal (1:1 composition) was observed in ethanol acetate buffer (pH 5, 0.2 M) by a spectrophotometric method and in the membrane system by the method of photoelectric measurement. This CTC had an association constant of 2.6 x 10^5 l/M, the energy of CT-band of 2.76 eV, and an extinction coefficient of 7450 l/Mcm in the solution system. The ionization potential of all-*trans*-retinal was evaluated as 8.47 eV. The light-induced potential developed across the bilayer lipid membrane containing all-*trans*-retinal was enhanced up to 570% at the presence of chloranil. This enhancement effect of chloranil was disscussed as a result of formation of the CTC with all-*trans*-retinal at the membrane interface, whose association constant was determined as 1.1 x 10^5 l/M. It was proposed that the membrane system could be utilized for screening the food-originated pesticides acting on energy-transfer processes and for elucidating their action mechanisms.

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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反応の定量的評価法

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要　　約

脂質二重膜における食料起源農薬の作用の定量的評価法を開拓するため，分光学的方法によりエタノール－酢酸緩衝液系（0.2M, pH5），および，光電位測定法により脂質二重膜系で，テトラクロルー1，4－ベンゾキノン（クロラニル）の全トランスーレチナール（組成比1：1）との電荷移動錯体（CTC）形成を検討した。このCTCは，溶液系で，結合定数が2.6×10^5 l/M，CT－帯のエネルギーが2.76eVで，吸光係数が7450l/Mcmであった。全トランスーレチナールのイオン化ポテンシャルは8.47eVであり，電位は，クロラニル存在下で，570%まで増加した。この増強効果は，結合定数が1.1×10^5l/MのCTCを膜表面において全トランスーレチナールと形成することによるものと考察した。この膜系が，エネルギー変換過程に作用する食料起源農薬のスクリーニング及びそれらの作用機構の解明に利用できることが提案された。

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