農薬生産用農産食料製造-全トランスーレチナールとアミノ化合物の付加反応(農芸化学科)

Pesticide-producing Agricultural Food Processing. Addition reaction of all-*trans*-retinal and amino compounds*

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I INTRODUCTION

In insect eyes, all-*trans*-retinal is generated by the action of light on rhodopsin, visual pigment[4]. An addition reaction of all-*trans*-retinal and amino compounds to form retinylidene compounds[1,2,8] was proposed by the author as one of the initial reactions resulting into the disturbance of visual functions in insects[5,6]. In the present work, in order to examine this proposition, the association constants of the addition reaction of all-*trans*-retinal and 22 amino compounds were evaluated and discussed from the structural aspects of the amino compounds used. The addition reaction was also investigated using the all-*trans*-retinal incorporated in bilayer lipid membrane (BLM)[9] in order to examine a possibility of the interaction at a membrane level, resulting into modification of the photopotentials generated accross BLM.

II MATERIALS AND METHODS

1 Chemicals

Amino acids were obtained from Ajinomoto Co., Ltd., Tokyo. All-*trans*-retinal was purchased from Eastman Kodak Company, Rochester, New York. Ferric chloride, iodine, aniline, and hydroxylamine, hydrochloride, were obtained from Wako Pure Chemical Industries, Ltd., Osaka. Other chemicals were in reagent grade as obtained from manufacturers.

2 Formation of retinylidene compounds

All-*trans*-retinal was dissolved into ethanol to make up 2×10^{-5} M solution. On the other hand, amino acids, aniline, and hydroxylamine, hydrochloride, were dissolved into double distilled water in excess to all-*trans*-retinal. A mixture of the above two solutions (10 ml: 10 ml) was brought to a strong alkali by addition of 0.5 ml of 2 N NaOH and was used for running absorption spectrum after standing for several hours. Next, a drop of concentrated HCl was added to

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* A part of this work was presented at the annual meeting of Agricultural Chemical Society of Japan, held in Kyoto on April 4th, 1976.

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Sci. Bull. Coll. Agr. Univ. Ryukyus, 27 : 129～138 (1980)

the preparation with vigorous shaking and utilized for running absorption spectrum immediately.

A reference cell contained distilled water. Reactions were carried out at a dark condition at a room temperature (25°C). All absorption spectra were obtained with a Beckman spectroscopy (Model DB-GT). Ion-free water was used throughout the present experiments.

3 Evaluation of association constant and molar extinction coefficient

1) Basic form

All-*trans*-retinal solution and amino acid solutions were prepared as described in the previous section. Keeping the constant concentration of all-*trans*-retinal solution, the concentrations of amino acid solution were adjusted to 1, 1/2, 1/4, 1/8, and 1/16 parts of the original concentration. From each amino acid solutions 10 ml was mixed with 10 ml of all-*trans*-retinal solution. To this mixture 0.5 ml of 2 N NaOH was added. The absorption spectra were obtained after standing the mixtures for various lengths of time. From the spectra obtained, the wavelength of a given system was determined when the peak shift became stable. And, at the same time, the lineality of the equation of Ketelaar and his coworkers[3] was tested. Then, from the equation, the association constant and the molar extinction coefficient of the retinylidene compound formed were calculated.

2) Acidic form

In a case of all-*trans*-retinal and aniline solution, an alkali treatment was not required and addition of a drop of concentrated HCl into the mixture (10 ml: 10 ml) with vigorous shaking was enough to observe spectral shift completion. For hydroxylamine, hydrochloride, a simple mixing with all-*trans*-retinal was enough for completing its spectral shift. The calculation of association constants and molar extinction coefficients was done as described above.

4 Membrane formation and photopotential measurement

Methods of forming the BLM containing all-*trans*-retinal and measuring the photopotentials induced by 0.5 sec illumination were the same to those described elswhere[7].

5 Evaluation of the effect of amino compounds on the photopotentials of all-*trans*-retinal BLM

1) Basic form

An aqueous phase was H_3BO_3·KCl-NaOH buffer (pH 9, 0.2 M). Electron acceptor was iodine dissolved in saturation in ethanol. After BLM formation, 0.04 ml of iodine solution was added into the outer solution (24 ml) being separated by BLM form the inner solution (8 ml). The photopotentials were obtained in intervals of 7 min. When photopotentials reached a stable value, an aliquout of the H_3BO_3·KCl-NaOH buffer (pH 9, 0.2 M) containing 0.5 M L-lysine was added to the inner solution. Then, the photopotentials were observed in intervals of 7 min.

2) Acidic form

Aqueous phase was sodium acetate buffer (pH 5, 0.2 M). Electron acceptor was ferric chloride dissolved in sodium acetate buffer (pH 5, 0.2 M). After BLM formation, an aliquot of the ferric

chloride solution was added into the outer phase to make up a concentration of 1×10^{-3} M. After mixing for 10 min and obtaining stable photopotentials, an aliquot of aniline or hydroxylamine solution was added into the inner phase. The photopotentials were observed in intervals of 7 min.

III RESULTS AND DISCUSSION

1 Retinylidene compounds

A simple mixture of all-*trans*-retinal and amino acids could not shift the absorbance peak of all-*trans*-retinal. An alkali treatment of the mixture resulted in a blue shift of the peak. Addition of a drop of concentrated HCl followed by vigorous shaking also shifted the new peak to a longer wavelength as shown in Fig. 1. In the case of L-lysine, the absorbance peak of all-*trans*-retinal

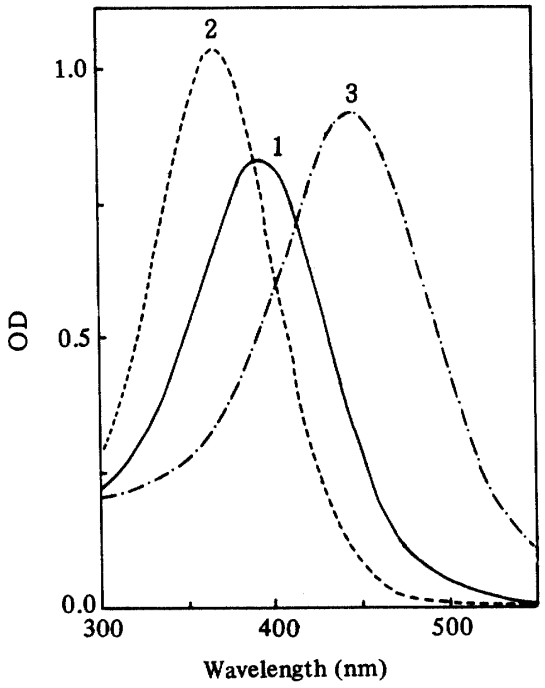

Fig. 1. Changes in absorption spectrum of all-*trans*-retinal and L-lysine dissolved in 50% ethanol water due to alkali and acid treatments
1 : All-*trans*-retinal (2.37×10^{-5} M) and L-lysine (2.25×10^{-2} M) dissolved in 50% ethanol water, 2 : After adding 2 N NaOH to 1, 3 : After adding a drop of concentrated HCl to 2

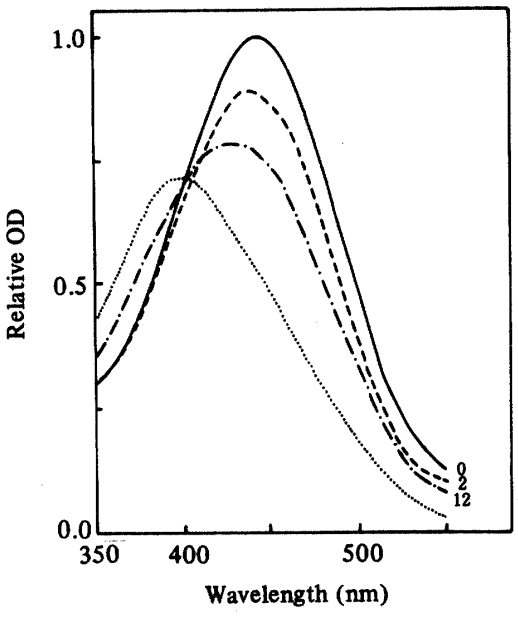

Fig. 2. The time dependence of absorption spectrum of all-*trans*-retinal and L-lysine in acidic 50% ethanol water
The numbers indicate the times after acidification in hr.

reappeared in 72 hr after the formation of the acidic form (Fig. 2). Other amino aicds, within 2 hr after the acidification, showed the absorbance peak of the respective acidic forms as shown in Table 1. The above observations indicated the formation of retinylidene compounds from all-*trans*-retinal and amino acids.

Table 1. The association constants, absorbance peaks, molar extinction coefficients, and reaction times of the addition products of all-*trans*-retinal and amino compounds

Amino acid	Association const. (1/M)	λ max (nm) acidic	λ max (nm) basic	Molar ext. coef. (1/Mcm)	React. time (hr)
Gly	120	368	438	45500	1
Ala	80	368	445	50400	0.5
Val	140	368	453	53000	1
Leu	180	369	455	50900	1
Ileu	160	369	455	51400	1
Phe	150	369	450	51300	0.5
β-Ala	560	365	445	47700	0.5
Ser	70	370	438	53000	2
Thr	70	370	449	48400	1
Cys-Cys	70	372	455	49600	60
Met	130	369	452	50600	1
Trp	140	370	454	50000	1
Asn	90	366	441	41500	2
Gln	50	370	446	49400	2
Asp	170	377	392	40500	2
Glu	90	370	448	50600	2
Lys	700	367	433	50600	1
Arg	90	368	450	53200	1
His	90	371	451	41500	2
Orn	1020	364	445	51700	1
Ha*	1500	-	360	45400	1
Anl**	210	390	500	53000	0.25

* Hydroxylamine, hydrochloride,
** Aniline

In an alkali treatment, the absorbance peak moved to a shorter wavelength as shown in Fig. 3 for a case of L-lysine.

In a case of aniline, not only a simple mixture of all-*trans*-retinal but also an alkali treatment could not shift its absorbance peak. An acid treatment, however, shifted the absorbance peak to 500 nm as shown in Fig. 4. In a case of hydroxylamine, hydrochloride, a simple mixing of the solutions resulted in formation of a new peak at 360 nm.

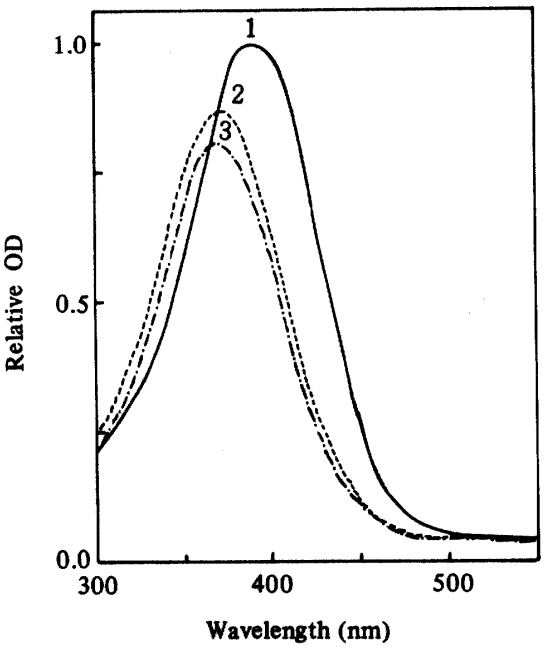

Fig. 3. The time dependence of the absorption spectrum of all-*trans*-retinal and L-lysine dissolved in alkaline 50% ethanol water
1 : Before NaOH addition, 2 : Half an hour after NaOH addition, 3 : One hour after NaOH addition

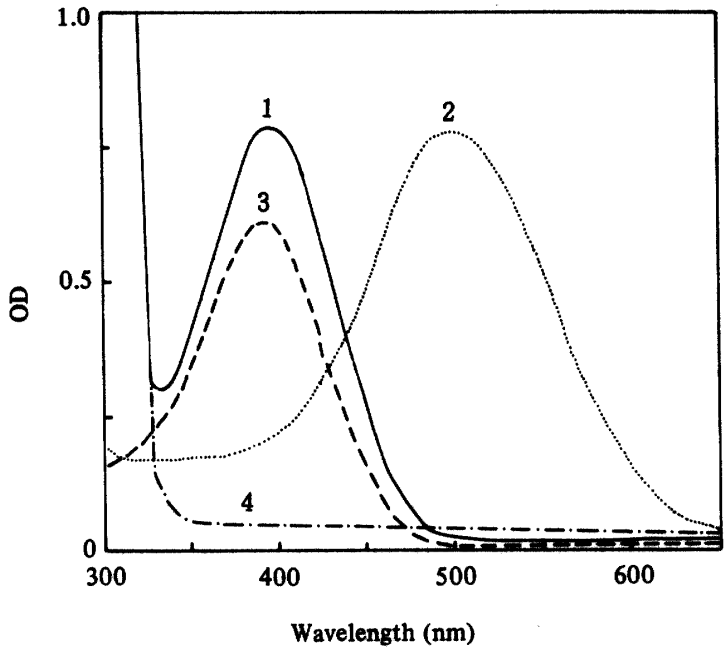

Fig. 4. Changes in absorption spectrum of all-*trans*-retinal and aniline dissolved in acidic 50% ethanol water
1 : All-*trans*-retinal (1.69×10^{-5} M) and aniline (0.47 M) dissolved in 50% ethanol water, 2 : After adding a drop of concentrated HCl to 1, 3 : All-*trans*-retinal (1.69×10^{-5} M) dissolved in 50% ethanol water, 4 : Aniline (0.47 M) dissolved in 50% ethanol water

From these observations, the association constants and molar extinction coefficients of retinylidene compounds were evaluated as basic forms for amino acids and an acidic form for aniline.

The equation of Ketelaar and his coworkers[3] well fitted the data obtained in the present work as shown in Fig. 5 for a case of L-lysine. The values determined by the spectrophotometric

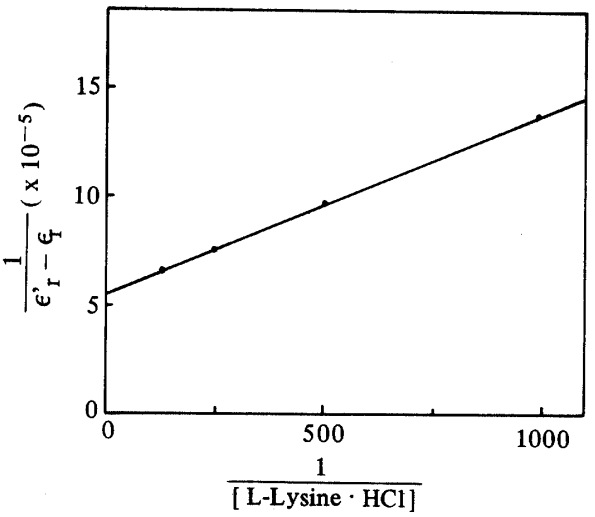

Fig. 5. The double reciprocal plot of the concentration of L-lysine, HCl, and the difference between the apparent molar extinction coefficient (ϵ'_r) and the molar extinction coefficient (ϵ_r) of all-*trans*-retinal at 367 nm for the method of Ketelaar and his coworkers

methods for all of the amino compounds used are shown in Table 1. The association constants of the retinylidene compounds formed between all-*trans*-retinal and amino acids varied from 70 l/M to 1020 l/M. The association constant of aniline was low while that of hydroxylamine was extremely high, about two times the value of L-lysine. The wavelengths of the absorbance peak varied from 360 nm to 377 nm for the basic forms and from 392 nm to 455 nm for the acidic forms. The molar extinction coefficients varied from 40500 l/Mcm to 43000 l/Mcm. The reaction time also varied from 0.5 hr to 60 hr.

Due to the relevance of association constants to the estimation of the reactivity with all-*trans*-retinal as vision inhibitors the relationship between the constant and the structure of amino acids was examined.

The number of amino groups contained in a given amino acid had no significance in relation to the value of association constants since L-ornithine and L-lysine, having two amino groups, had high values while L-arginine and L-glutamine had low values and β-alanine, having one amino group, had a high value while other amino acids with one amino group had lower values.

The next comparison was made on the acidity and basicity of amino acids. Acidic amino acids showed lower values. Neutral amino acids had values around 100 l/M while β-alanine had a higher value. Among basic amino acids L-lysine and L-ornithine had higher values while L-arginine, L-histidine, and others had low values. From these points, there seemed to be no relationship between the acidity or basicity of the side chain and the association constant.

On the other hand, the pK values of the amino group of the amino acids used had a paralel relationship at least with those having higher association constants. That is, the order of associ-

ation constants, L-ornithine, L-lysine, and β-alanine, coinsided with the order of pK values of δ-amino group of L-ornithine, ϵ-amino group of L-lysine, and β-amino group of β-alanine. With the other amino acids having the lower pK values of the amino group involved did not show such a clear tendency, e.g. L-aspartic acid showed 2.2 times the association constant of β-alanine inspite of similar pK values, indicating that some factors were involved in the formation of retinylidene compounds beside the pK values of the amino group concerned.

The formation of retinylidene compounds with the amino acids which can be found in the food materials processed from agricultural products may be considered as one of the mechanisms of the vision inhibitors having the safety guaranteed. The pK value of the amino group of amino acids as well as the association constant may be utilized as an indicator of the inhibitory action especially at a molecular level.

2 Membrane-potential modification

L-Lysine among the amino acids used was selected for BLM work because of its higher association constant and shorter reaction time required. In the following BLM experiments, L-sysine, aniline, and hydroxylamine were used as membrane modifiers.

The effect of L-lysine on the photopotential of BLM containing all-*trans*-retinal was observed as a decrease in the photopotential as much as 85% by addition of 0.3 M L-lysine into the inner chamber.

In contrally to L-lysine modification of the photopotential, the addition of aniline solution into the inner chamber increased the photopotentials upto 350% of the unmodified photopotentials at a concentration of 0.1 M.

The addition of hydroxylamine into the inner chamber decreased the value of the photopotentials as much as 40% of the unmodified photopotentails at 2×10^{-4} M.

It is interesting that the reductive or enhansive effect of the additives was similar to the spectral shift due to the addition reaction of all-*trans*-retinal and the amino compounds; hypsochromic shift and bathochromic shift resulted in a reduction and an enhancement, respectively. Since the state of the molecular organization at the BLM interface can be assumed as shown in Fig. 6, the above observations may indicate that the spectroscopic properties of the pigments formed at the interface may determine the magnitude of the photopotentials generated. Although further works are required for characterizing the dynamic actions of amino compounds on the BLM, it may be said that the BLM system can be applied for screening the possible inhibitors related to amino compounds.

The author has proposed that the formation of retinylidene compounds with amino acids could be considered as one of the primary reactions leading to the disturbance of insect vision[5,6]. As shown in Fig. 7, the action would come from L-cysteine (c)[6], and other amino acids (d) by shifting the absorbance peak of photo-generated all-*trans*-retinal (e) toward the insect-selective spectral range (b – a)[6]. On the other hand, the action of substances like aniline (f) would cause a new type of disturbance by modifying visual sensitivity to visible light.

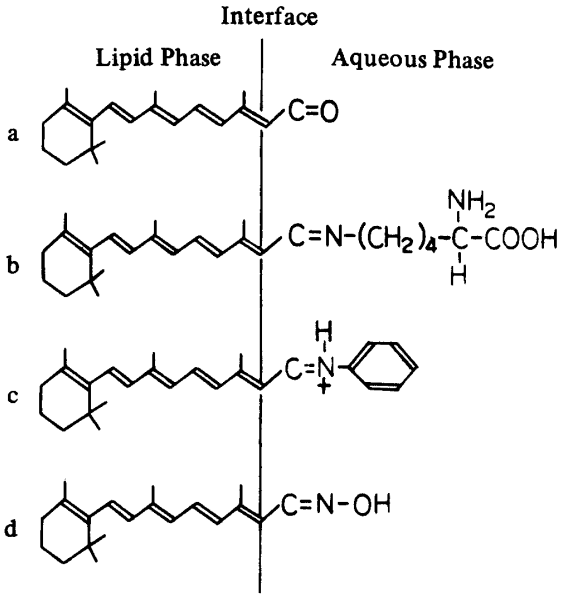

Fig. 6. A schematic diagram showing the pigment orientation at the interface of BLM after an addition reaction of all-*trans*-retinal (a) with L-lysine (b), aniline (c), and hydroxylamine (d)

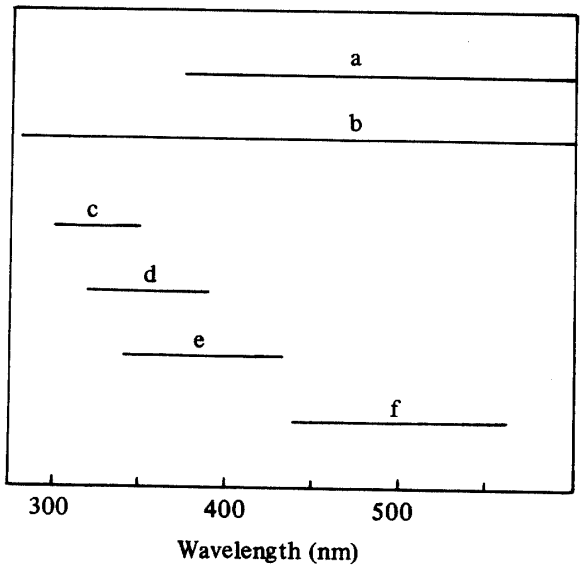

Fig. 7. A diagram showing the spectral ranges of the light transmitted into vertebrate eyes (a) and insect eyes (b) and the half absorption band widths of all-*trans*-retinal (e) after an addition reaction with L-cysteine (c), L-lysine (d), and aniline (f)
The data shown as a, b, and e were quoted from the work reported elswhere[6].

IV SUMMARY

The association constants and molar extinction coefficients of the product of the addition reaction of all-*trans*-retinal and 20 amino acids were determined. Those having the higher pK values of amino group, such as L-ornithine, L-lysine, and β-alanine, gave greater association constants, indicating not only the association constant of retinylidene-compound formation but also the pK value of the amino group involved in the reaction would be utilized as an indicator for the purpose of screening amino-acid related, food-originated insect-vision inhibitors at a molecular level. For aniline and hydroxylamine, the association constants and molar extinction coefficients of the respective product formed were also determined. L-Lysine, aniline, and hydroxylamine modified the photopotentials generated across all-*trans*-retinal bilayer lipid membranes in appropriate conditions, showing the applicability of the membrane system for screening the insect-vision inhibitors having amino groups.

ACKNOWLEDGEMENT

The author is thankful to Misses Y. Kinjo and N. Oshiro for their technical assistance.

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農薬生産用農産食料製造—全トランス-レチナールとアミノ化合物の付加反応

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要　約

全トランスーレチナールと20種のアミノ酸の付加反応は，50%エタノール水中で行なわれ，その結合定数と生成物のモル吸光係数を求めた。使用したアミノ酸の中で，アミノ基のpK値の高いL－オルニチン，L－リジン，及びβ－アラニンが高い結合定数を与えた。このことから，アミノ酸系の食料起源の昆虫視覚阻害剤の分子レベルに於けるスクリーニングには，レチニリデン化合物形成反応の結合定数と共に，反応に関与するアミノ基のpK値が指標となり得ることが示された。アニリン及びヒドロキシルアミンについても，結合定数と反応生成物のモル吸光係数を求めた。L－リジン，アニリン，及びヒドロキシルアミンが，適切な条件下で，全トランスーレナチール脂質二重膜の光電位発生に影響を及ぼすことを認め，同膜系が，アミノ基を持つ昆虫視覚阻害剤のスクリーニングに利用できることが示された。

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