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Binding of kynurenine to catecholamine

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Summary. Papiliochrome II is a pale yellow pigment of butterflies and consists of one molecule each of *L*-kynurenine and N-β-alanyldopamine (NBAD). The aromatic amino nitrogen of kynurenine is bonded to the β-carbon of NBAD. There are isomers IIa and IIb which show opposite circular dichroism. The β-alanine contents of IIa and IIb were determined and the molar ratio of IIa to IIb has proved to be 1.17. The IIa and IIb were decomposed to *L*-kynurenine and N-β-alanylnorepinephrine (NBANE) by being heated in water at 80°C for 30 min. In both IIa and IIb, circular dichroism of the NBANE showed the same positive peak at 280 nm. The NBANE were further decomposed to β-alanine and norepinephrine (NE) by being heated in 1 N HCl at 100°C for 2 hr. The NE was submitted to enantioseparation and has proved to be a racemic mixture in both cases of IIa and IIb. These results are discussed in the light of the enzymic synthesis of IIa and IIb.

Keywords: Amino acids – Papiliochrome II – Kynurenine – N-β-Alanyldopamine – N-β-Alanylnorepinephrine – Norepinephrine

Introduction

Kynurenine is an intermediate of tryptophan metabolism and an aromatic amine. On the other hand, catecholamines are *o*-diphenolic metabolites of tyrosine and include neurotransmitters and hormone. If there exists a compound consisting of both kynurenine and catecholamine, it will be interesting in that the compound is related to both tryptophan and tyrosine metabolism. Papiliochrome II, a pale yellow pigment in the wing-scales of papilionid butterflies, is the very compound of such combination. Chemical properties of the pigment has been reviewed by Umebachi (1985, 1990).

Papiliochrome II consists of one molecule each of *L*-kynurenine and N-β-alanyldopamine (NBAD). The latter compound is a new catecholamine which was discovered in the course of the investigation of Papiliochrome and is now known to be widely distributed in insects (Kramer and Hopkins, 1987; Sugumaran et al., 1990; Andersen, 1990).

Papiliochrome II readily decomposes to *L*-kynurenine and N-β-alanylnorepinephrine (NBANE) under mild conditions (in water at 80°C for 30 min). And the NBANE further docomposes to β-alanine and norepinephrine (NE) by being heated in 1 N HCl at 100°C for 2 hr (Umebachi and Yamashita, 1976, 1977; Rembold et al., 1978). From ^{13}C-NMR and Mass spectra, the structure of Papiliochrome II has been reported to be N^{ar}-[α-(3-aminopropionylaminomethyl)-3,4-dihydroxybenzyl]-*L*-kynurenine, in which the aromatic amino group of kynurenine is bonded to β-carbon of the side chain of NBAD (Fig. 1) (Rembold and Umebachi, 1984).

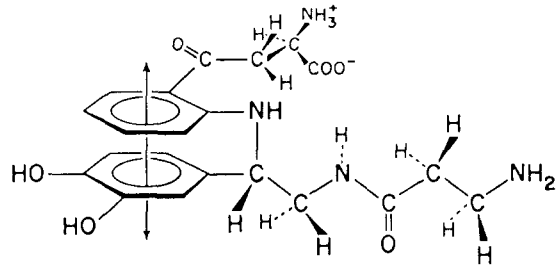

Fig. 1. Structure of Papiliochrome II (Rembold and Umebachi, 1984)

In Papiliochrome II, interestingly, there are two isomers named IIa and IIb, which can be separated by cellulose thin-layer chromatography and give opposite optical rotatory dispersion (ORD) and circular dichroism (CD) (Umebachi and Yoshida, 1970). In the structure shown in Fig. 1, the configuration around α-carbon in the side chain of kynurenine is of *L*-form in both IIa and IIb. Therefore, the IIa and IIb are not enantiomorphic to each other. As will be seen in the structure of Fig. 1, there is another asymmetric carbon atom. It is the β-carbon atom in the side chain of NBAD. The configuration around this β-carbon in IIa and IIb has remained to be studied.

Yago (1989) and Yago et al. (1990) succeeded to enzymatically synthesize Papiliochrome II. According to them, when *L*-kynurenine and NBAD were incubated with phenoloxidase from mantids, Papiliochrome IIa and IIb were produced. Sugumaran et al. (1990) and Saul and Sugumaran (1991), too, synthesized Papiliochrome IIa and IIb from *L*-kynurenine and NBAD using phenoloxidase of *Sarcophaga*. According to them, the mechanism of the enzymic synthesis of IIa and IIb is as shown in Fig. 4. First, NBAD (I) is oxidized to NBAD *o*-quinone (II) by phenoloxidase. The NBAD *o*-quinone is converted to its tautomer, NBAD quinone-methide (III). Although this reaction occurs spontaneously a little, there is an isomerase which catalizes this reaction in insects. To the β-carbon of the side chain of NBAD quinone methide thus formed, the aromatic amino nitrogen of *L*-kynurenine is attached. It is important that this reaction occurs non-enzymatically. And there is a strong possibility that the products IIa and IIb are isomers of configuration around the β-carbon in the side chain of NBAD. Therefore, probably the IIa and IIb are diastereomers as the whole molecule (Saul and Sugumaran, 1991). Thus, the NBANE which is produced by the decomposition of IIa or IIb must be (+) or (−) around the β-carbon.

The purpose of the present paper is to examine the configuration of the NBANE and NE produced by the decomposition of IIa and IIb.

Materials and methods

Materials

The pale yellow scales in the wings of *Papilio xuthus* and *Papilio demoleus* (Papilionidae: Lepidoptera) were used. The main pale yellow pigments from both species are Papiliochrome IIa and IIb.

Extraction and separation of the pigments

The pale yellow pigments were extracted from the scales with 70% ethanol at 25°C. After centrifugation at 3000 rpm for 10 min, the extract is pale yellow and includes almost all of Papiliochrome. As Papiliochrome II is unstable to heating, the above extract was, without any pretreatment, submitted to one-dimensional thin-layer chromatography (TLC) with cellulose plate (Merck, No. 5716, 20 × 20 cm). After developing with a mixture of *n*-butanol, water and acetic acid (120:50:30 v/v) (BAW), the chromatogram was inspected under ultraviolet rays. Papiliochrome IIa and IIb can be separated by this TLC and can be detected by their pale yellow fluorescence on the chromatogram. The areas of IIa and IIb were scraped separately and stored in a cold vacuum desiccator. The cellulose powder thus obtained is below called the starting material.

UV and CD spectra of NBANE

Papiliochrome IIa or IIb was extracted from the above starting material with 70% ethanol at 25°C. From the extract, ethanol was removed below 25°C in rotary evaporator and the remaining water solution was lyophilyzed. The lyophilyzed residue was dissolved in water and sealed in vacuum tube. After being heated at 80°C for 30 min, the content was again lyophilyzed. The lyophilyzed residue was dissolved in 70% ethanol and submitted to TLC with the above cellulose plate. After developing with BAW, the marker strips 1 cm from the both side of plate were cut out and submitted to the 2% phosphomolybdic acid-ammonia test for phenolic compounds (Riley, 1950). As NBANE is positive to this reaction, the area of NBANE was scraped from the remaining plate and extracted with 70% ethanol at 25°C. From the extract, ethanol was removed below 25°C in rotary evaporator and the remaining water solution was further lyophilyzed. The lyophilyzed residue was dissolved in 70% methanol, and its UV and CD spectra were taken with the Shimadzu UV-265FS spectrophotometer and the JASCO spectropolarimeter J-40C, respectively.

Separation of (±)norepinephrine

The above NBANE was again lyophilyzed, dissolved in 1 N HCl, and sealed in vacuum tube. After being heated at 100°C for 2 hr, the content was diluted with water and lyophilyzed. The separation of (±)NE was performed by either of the following two methods. (1) The above lyophilyzed residue was dissolved in $HClO_4$ water solution (pH 1.5) and applied to the Crownpack CR(+) column (4.0 × 150 mm, Daiseru Co.) and eluted at 0–2°C with the same $HClO_4$ solution at a flow rate of 0.5 ml per min. With this column, (−) and (+)NE can be separated. The effluent was monitored with the absorption at 280 nm. (2) The above lyophilyzed residue was dissolved in a little of methanol and mixed with a few drops of salicylic aldehyde. By this procedure, the Schiff's base is formed. The yellow solution thus obtained was submitted to TLC (Merck, precoated HPTLC plate CHIR, No. 14285, 10 × 10 cm) and developed with a mixture of chloroform and methanol (90:10 v/v), 80% saturated with water. By this TLC, (−) and (+)NE can be separated. The spots can be detected with visible rays or under ultraviolet ray at 260 nm.

Determination of beta-alanine

In order to decide the ratio between IIa and IIb, the IIa or IIb was extracted from the starting material with 70% ethanol at 25°C, and the extract was evaporated to dryness below 30°C in rotary evaporator. The residue was dissolved in 6 N HCl, and sealed in vacuum tube. After being heated at 106°C for 16 hr, the hydrolysate was diluted with water and evaporated to dryness at 60°C in rotary evaporator. The residue was dissolved in water and submitted to amino acid analyzer (Shimadzu LC-4).

Results

Ratio of IIa to IIb

The molar ratio between isomers IIa and IIb was decided by determining the β-alanine from IIa and IIb. The molar ratio of IIa to IIb was 1.17.

UV and CD spectra of the NBANE from IIa and IIb

The UV absorption spectra of NBANE are given in Figs. 2a and b, which show that the absorption peak is at 280 nm in both cases of IIa and IIb and indicate that the compounds from IIa and IIb are catechol derivative but not ketocatechol. The CD spectra of the NBANE from IIa and IIb are given in Figs. 2c and d, which show that the NBANE from IIa and IIb both have a peak of CD at 280 nm and in the same positive side.

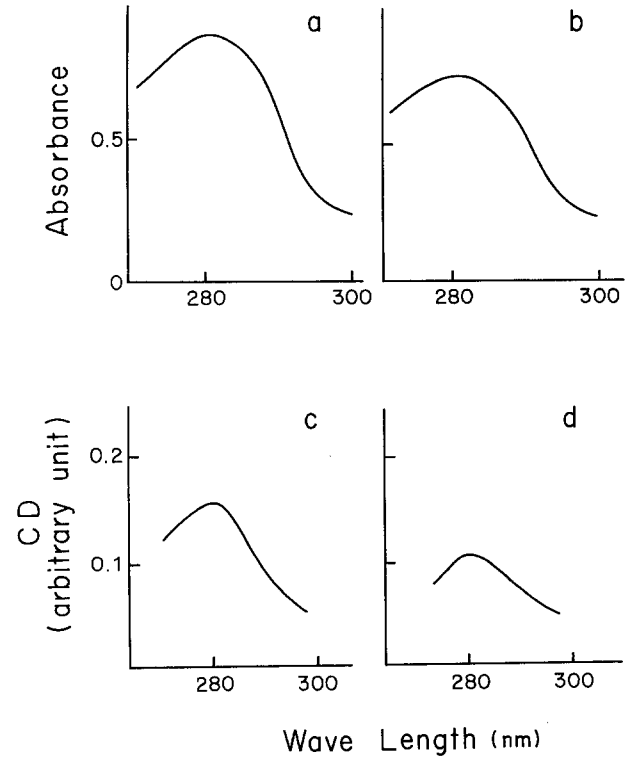

Fig. 2. UV- and CD-spectra of the N-β-alanylnorepinephrine produced from IIa and IIb. **a** and **b** UV-spectra from IIa and IIb, respectively. **c** and **d** CD-spectra from IIa and IIb, respectively

Separation of (±)NE

The NE released by hydrolysis of the NBANE from IIa or IIb was submitted to HPTLC and HPLC for the separation of enantiomers. In both cases of IIa and IIb, both (−) and (+)NE were detected in both HPTLC and HPLC. The elution patterns from the HPLC column are given in Fig. 3, which shows that there is not a quantitatively big difference between (−) and (+) produced from IIa or IIb, though the quantity of (−)NE was a little larger than (+)NE.

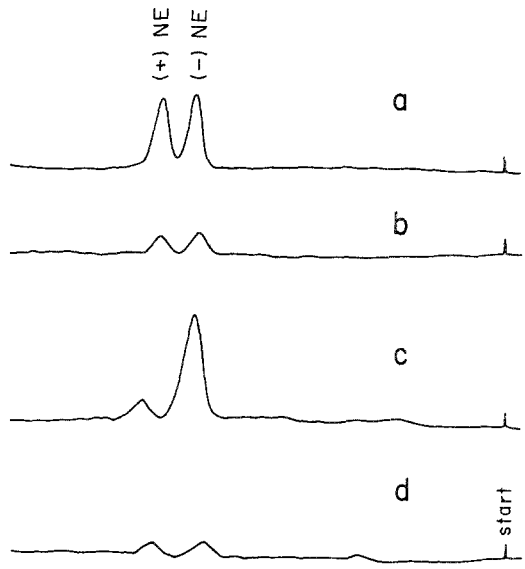

Fig. 3. Separation of norepinephrine (NE) enantiomers with HPLC. **a** authentic (±)NE; **b** the NE from IIa; **c** the NE from IIa plus authentic (−)NE; **d** the NE from IIb

Discussion

As already reported by Umebachi and Yoshida (1970), Papiliochrome IIa and IIb give opposite ORD and CD spectra in the wave length range of 250 to 450 nm. But the IIa and IIb are not enantiomorphic to each other, because kynurenine is of *L*-form in both IIa and IIb.

In the structure of IIa and IIb, there is another asymmetric carbon atom. It is the β-carbon of the side chain of NBAD. Between IIa and IIb, is the configuration around the β-carbon atom of NBAD the same or opposite? The fact that the NBANE released from IIa and IIb in water at 80°C for 30 min both gave the same positive peak of CD suggests that the configuration around the β-carbon atom is the same in both IIa and IIb.

From the mechanism of the enzymic synthesis of Papiliochrome IIa and IIb, on the other hand, the aromatic amino nitrogen of kynurenine is probably non-enzymatically bonded to the β-carbon atom of the side chain of NBAD (Saul and Sugumaran, 1991). Therefore, the configuration around the β-carbon atom of NBAD should be opposite between IIa and IIb (Fig. 4).

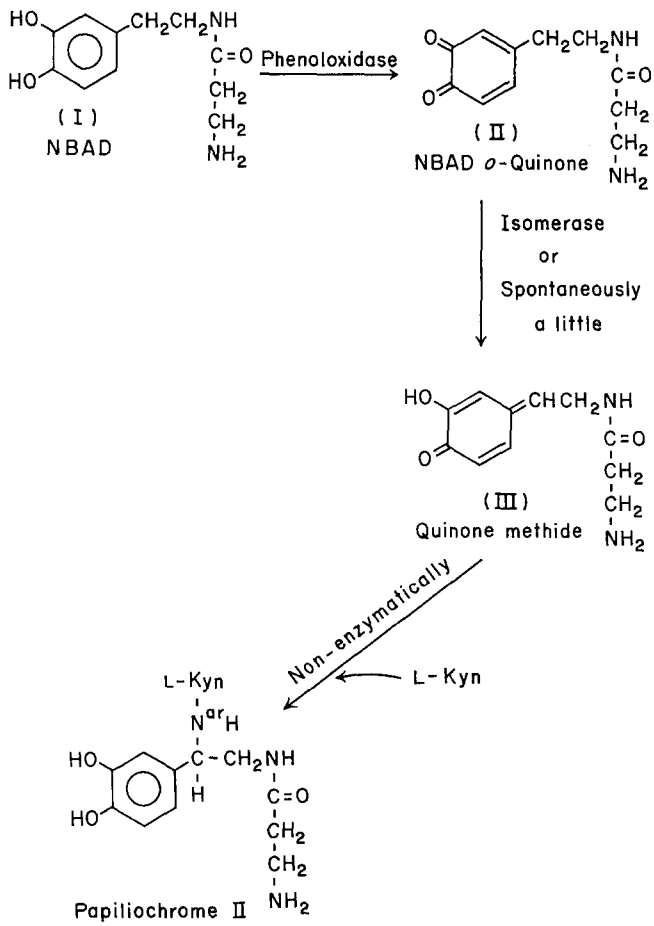

Fig. 4. Proposed mechanism of the enzymic synthesis of Papiliochrome II (From Saul and Sugumaran, 1991)

Thus, the experimental results of the present paper and the theory from the synthesis of IIa and IIb are in conflict with each other. One possibility is that, when NBANE is formed by the split of IIa (or IIb), the configuration around the β-carbon atom of NBAD become one-sided. But this point has remained unsettled.

It is interesting that the NE produced by acid hydrolysis of NBANE is a racemic mixture. However, as authentic (−)NE is readily racemized by being heated in dilute HCl, there is a possibility that the racemic NE separated in the present paper resulted from racemization of (−) or (+). Therefore, in not only NE but also NBANE, the heating in HCl must be avoided at least for the research of configuration, though it is difficult to get NE from NBANE without hydrolyzing in HCl. This problem has also remained unsolved.

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