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Flavin-photosensitized Production of Indole-3-acetaldehyde from Tryptophan

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Abstract: Photochemical reaction of tryptophan with flavin was performed under anaerobic condition to give indole-3-acetaldehyde as a major product.

In plants, indole-3-acetic acid (IAA), a natural auxin, has been generally accepted to be biosynthesized from tryptophan (Trp)[1]. In the course of our attempts to identify the pathway of IAA biosynthesis in plants, we found a novel system for the formation of IAA from Trp in extracts of maize coleoptiles[2]. The IAA-forming activity was co-purified with indole-3-acetaldehyde (IAAld) oxidase (aldehyde oxidase). We also found that FAD (flavin adenine dinucleotide) or FMN (flavin mononucleotide) had a non-enzymatic activity to produce IAAld from Trp. Aldehyde oxidase of animals is known to have two FAD as a prosthetic group[3]. Many reports dealing with production of *N*-formylkynurenine and its derivatives from Trp have been accumulated with riboflavin or methyleneblue as a photosensitizer in the presence of oxygen molecules[4], and also with gamma- or X-irradiation in aqueous solution[5], but the formation of IAAld from Trp with flavin has not been reported. In the present study, we

show that IAAld is photochemically produced with flavin under anaerobic condition, and discuss the possible function of FAD in aldehyde oxidase on Trp metabolism in plants.

As shown in Fig. 1, a major product was observed after light irradiation for 60 min with FMN under anaerobic condition (Fig. 1-b). The product had the same retention time as that of authentic IAAld and was identified as IAAld by gas chromatography-mass spectrometry (GC-MS) analysis[6]. Under aerobic condition the production of IAAld was less than that produced under anaerobic condition, and additional other peaks were detected (Fig. 1-c).

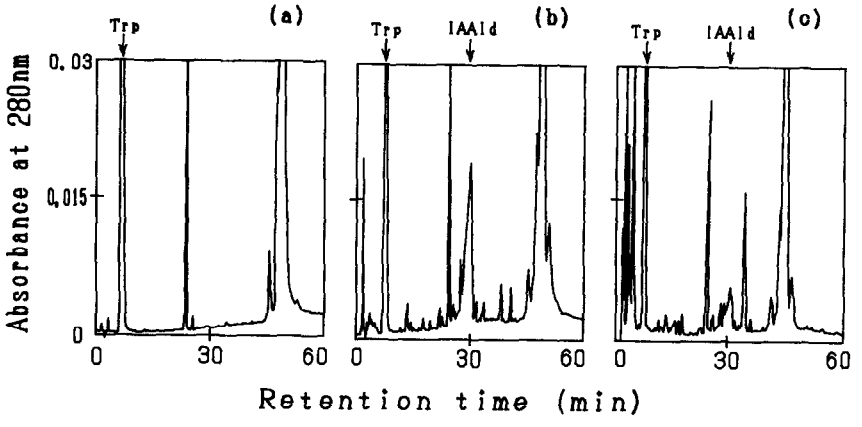

Fig.1 HPLC analysis of reaction products of Trp with FMN under anaerobic and aerobic conditions. Test samples which are composed of 2.5 μmoles of L-Trp and 250 nmoles of sensitizer in 1 ml of purified water (Milli-Q-SP) were placed at a distance of about 3 cm from a fluorescent light source (20-W, Hitachi FL-20-SD-G) for 60 min. The mixtures were bubbled by O_2 or N_2 gas during the light irradiation. A portion (100 μl) of the test solution was analyzed by high performance liquid chromatography (HPLC). HPLC was performed with an ODS C-18 column (Tosoh, 0.5 x 15 cm) at a flow rate of 1 ml/min. The solvent system was a 10-50% methanol gradient containing 0.2% acetic acid. (a) 0-Time control; (b) after photoreaction under anaerobic condition for 60 min at 28°C; (c) after photoreaction under aerobic condition for 60 min at 28°C. A large-scale reaction was carried out to identify the product by GC-MS. The reaction mixture (20 ml) containing 5 mM L-Trp and 250 μM FMN was incubated for 60 min as described above. The acidic and neutral materials were partitioned to ether phase (10 ml x 3). After ether was removed by N_2 blowing, the residue was dissolved in 150 μl methanol and analyzed by GC-MS.

Riboflavin and FAD also photochemically produced IAAld (Table 1). Under aerobic condition a large decrease in amount of Trp was observed whereas the production of IAAld was low. Other type of sensitizer (methylene blue) producing singlet oxygen and gamma-irradiation producing hydroxyl and superoxide radicals caused similar or higher decrease of Trp, but no IAAld production was detected. Thus, it is likely that Trp is easily oxidized by oxidizing species such as singlet oxygen, hydroxyl and superoxide radicals to form products including *N*-formylkynurenine as a major component. In contrast, under anaerobic condition with flavin IAAld is photochemically produced as a major component.