A Novel Mushroom Pigment: Isolation and Characterization

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Since our discovery of an oxygenase-type conversion of tryptophan to kynurenine[1] (which was made before discovery of oxygenases[2,3]), our interest has extended to the elucidation of biological functions of pigments existing in living systems.[4,5] We have been particularly interested in the biological function of mushroom pigments because this area remains unexplored, and we have undertaken the first investigation on the isolation and characterization of a pink pigment in the edible mushroom, *Pleurotus Salmoneostramineus* L. Vass. Although this mushroom was found in the inshore county of Siberia in 1973, it is now known to be widely distributed in Russia, Japan, New Guinea, and elsewhere.[6,7] Both the face and the back of this mushroom's cap assume a beautiful pink color. However, it ages around 1 week after germination and becomes whitish. Therefore, the mushroom was used for the investigation[8] 3 days after germination.

The pigment was isolated from the mushroom chromoprotein[9] which was purified by repeated gel filtration (on Sephadex G-50) of the water extract of the well-ground mushroom. Upon addition of acetone (or methanol) to the aqueous chromoprotein solution (pink color), the solution turned orange and a white solid (glycoprotein containing metals)[10] was precipitated. Careful evaporation of acetone (or methanol) from the filtrate provided the pigment as crystals. Its structure was determined by the following spectroscopic (UV–vis, IR, MS, and NMR) analyses. The pigment crystals were dark reddish-orange prisms, mp 110–112 °C dec. The R_f value was 0.14 on silica gel TLC (AcOEt/hexane = 1/1). UV–vis λ_{max} (MeOH, nm) (log ϵ): 208 (3.12), 220 (3.11), 260 sh (2.76), 282 (2.70), 290 (2.69), 300 sh (2.65), 430 sh (2.79), 456 (2.84) and 480 sh (2.75). The characteristic bands are shown in Figure 1. The IR ($CHCl_3$) spectrum suggests the presence of C=O (ν_{max} 1711 cm^{-1}, st), C=N (1651, st), and C=C (1603, st). The molecular formula C_8H_5NO was determined by the fast atom bombardment mass spectrometry [FAB-MS] positive (m/z 132 $[M + H]^+$) and negative (m/z 130 $[M - H]^-$) ionization methods (glycerol matrix) and exact FAB-MS spectra (found m/z 132.0470; calcd for C_8H_6NO, $[M + H]^+$, m/z 132.0449). The 500 MHz ^{1}H-NMR (CD_3OD) spectrum showed signals in the aromatic region at δ 7.04 (td, J = 8.0, 7.0, 1.0 Hz, H-5), 7.12 (td, J = 8.0, 7.0, 1.0 Hz, H-6), 7.20 (bs, H-2), 7.36 (td, J = 8.0, 1.0, 0.5 Hz, H-7), and 7.69 (td, J = 8.0, 1.0, 0.5 Hz, H-4).[11] From these spectroscopic data, the chemical structure of the pigment was elucidated as 3*H*-indol-3-one (**1**, indolone), which is a new $4n\pi$ (n = 2) aromatic system. The structure of the pigment was also confirmed by its chemical synthesis.[12] The electron affinity and ionization potential for **1** were calculated to be −1.36 and −9.90 eV, respectively, by the MNDO-PM3 method.

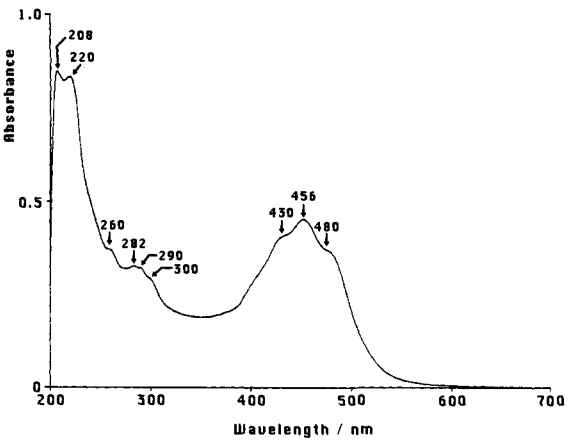


Figure 1. UV–vis spectra of the pigment (indolone) in MeOH. Concentration, 0.085 mg/mL; length of the cell, 1 cm.

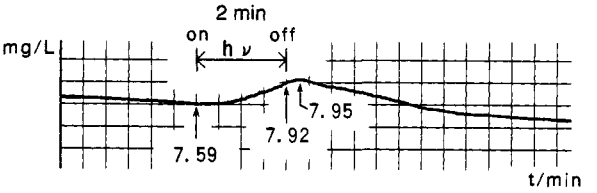
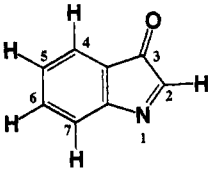


Figure 2. Evolution of oxygen by intermittent irradiation of the aqueous chromoprotein solution in the presence of the protein with β-structure at ambient temperature. Chromoprotein, 10 mg; protein with β-structure, 20 mg; aqueous solution, 13 mL; pH, 6.93 at 24 °C; light source, tungsten lamp (45 W); values attached to the arrow, amount of dissolved oxygen (mg/L) measured by Horiba OM-14.



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Indolone decomposes slowly in solution at ambient temperature but is stable in its crystalline form. It is extremely stable in the chromoprotein even in aqueous solution, which can be stored for several months without decomposition. Indolone is also stabilized by its formation of an inclusion complex with α-cyclodextrin, whose absorption maximum appears at 473 nm. A larger

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(8) The mushroom was cultivated by one of the authors (K.I.) at Kinki University and also by N. Kotera at Sumitomo Chemical Co.

(9) The pink pigment purified by repeating gel filtration was identified as a chromoprotein with an α-helix structure. This consists of the pigment (indolone), a glycoprotein with a galactose chain, and three metals (Zn, Fe, and Cu). Gel filtration also provided a pale yellow glycoprotein with β-structure.

(10) The white glycoprotein contains three metals (Zn, Fe, and Cu).

(11) The 500 MHz ^{1}H-NMR spectra of **1** in D_2O/acetone-d_6 (1/2) are as follows: δ 7.04 (td, J = 8.0, 7.0, 1.0 Hz, H-5), 7.13 (td, J = 8.5, 7.0, 1.0 Hz, H-6), 7.29 (bs, H-2), 7.44 (td, J = 8.5, 1.0, 0.5 Hz, H-7), and 7.61 (td, J = 8.0, 1.0, 0.5 Hz, H-4).

(12) The chemical synthesis of 3*H*-indol-3-one will be reported elsewhere.

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bathochromic shift (40 nm) was observed in the absorption spectrum (λ_{max} 496 nm) of the chromoprotein reconstituted from indolone (λ_{max} 456 nm) and the white glycoprotein containing metals (Zn, Fe, and Cu). These results suggest some specific interaction between them.

We observed the generation of oxygen (Figure 2) when an aqueous solution of the chromoprotein and the glycoprotein with β-structure[9] was intermittently irradiated with a tungsten lamp (45 W) at ambient temperature. As shown in Figure 2, an induction period is seen in the photochemical generation of oxygen. Oxygen evolution was clearly observed, even after a fifteenth irradiation of the solution. Without indolone, no photochemical generation of oxygen was observed with either glycoprotein.

Thus, it is concluded that the indolone in these proteins plays a very important role in the photochemical generation of oxygen from water, suggesting the possibility of the involvement of indolone in photosynthesis. Intensive investigation is currently underway to elucidate the structure of the proteins and to reveal the dark reactions in this system.

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