TRANSFORMATION OF HORSERADISH PEROXIDASE INTO PHOTOSENSITIVE COMPOUNDS IN THE PRESENCE OF INDOLE-3-ACETIC ACID

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Received May 20, 1968

A considerable amount of attention has been given to the reaction of indole-3-acetic acid (IAA) with phyto-peroxidases, (Ray 1958). But the attention was mainly focused on the degradation of IAA. IAA is oxidized by pure peroxidase under aerobic conditions without added hydrogen peroxide. Major end-products of IAA oxidation were identified as indole-3-aldehyde and 3-methylene oxyindole (Hinman and Lang, 1965; Morita, 1967). Of the various hydrogen donors in the horseradish peroxidase (HRP) reaction, IAA is considered to be a peculiar one from the following reasons. 1. HRP is reduced effectively by an intermediate (possibly IAA free radical) formed in the peroxidatic oxidation of IAA (Yamazaki and Piette, 1963). 2. HRP is denatured to be precipitated in the presence of IAA and hydrogen peroxide under strictly anaerobic conditions (Yamazaki and Souzu, 1960). 3. IAA reacts with oxygenated HRP (Compound III) at a considerable rate (Yokota and Yamazaki, 1965). 4. An isozyme of HRP is converted into so-called "Compound IV" during oxidizing IAA under aerobic conditions (Yokota and Yamazaki, 1965). The degradation

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of HRP hemin under physiological conditions seems very interesting and will be reported in this paper.

Chance (1949) reported that in the presence of excess methyl peroxide peroxidase existed in the form of Compound IV which was a verdo compound with an absorption band at 675 mμ and no enzymic activity. Compound IV formation occured only under non-physiological conditions and little work has been done since that time. We noticed that a certain isozyme of HRP was converted into a product similar to Compound IV during aerobic oxidation of IAA catalyzed by the enzyme itself (Yokota and Yamazaki, 1965). This isozyme was purified from CM-cellulose adsorbed fraction and was later found to correspond to Isozymes B and C in the classification of HRP by Shannon et al (1966). The enzyme is almost completely converted into P_{670} compound after several additions of IAA under aerobic conditions as shown in Fig. 1. The efficiency of P_{670} formation

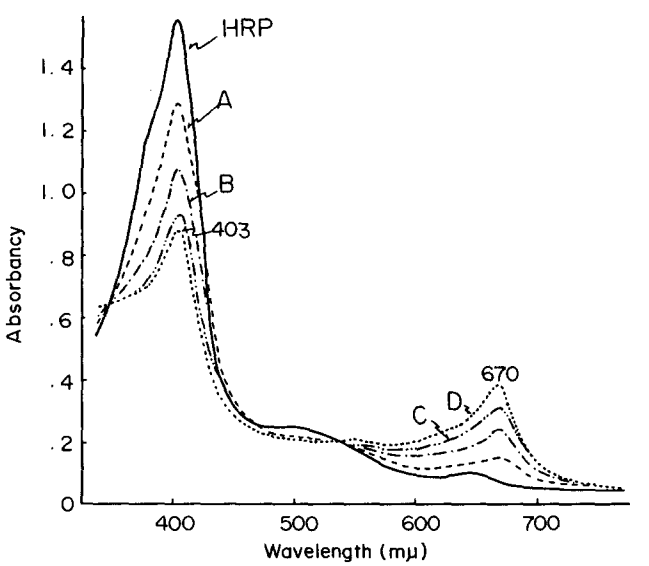

Fig. 1. Transformation of HRP into a verdohemoprotein in the presence of IAA under aerobic conditions 15.5 μM HRP, 0.05 M acetate, pH 4.0, 20°. A; a few minute after an addition of 0.1 mM IAA to HRP solution. B, C and D; a few minute after 2nd, 3rd and 4th additions of 0.1 mM IAA to the solutions of A, B and C, respectively.

is parallel to the velocity of IAA oxidation and highest at pH 4.0. P_{670} is a green compound and similar to an intermediate in the transformation from hemoglobin to biliverdin which has been reported in the model system (Lemberg and Legge, 1949; Kajiro and Kikuchi, 1951) and in the enzymic system (Nakajima et al, 1963). P_{670} can be easily isolated from IAA oxidation products and a small amount of unreacted HRP by the Sephadex and CG-50 column chromatographies. The purified P_{670} is very stable and its optical absorption spectrum is shown in Fig. 2. A spectrum of pyridine hemichrome of P_{670} is also demonstrated in Fig. 2, which indicates that the chromophore of P_{670} may be equal to an oxidized product of pyridine hemichrome in the presence of heme α-methenyl oxygenase and NADPH (Nakajima, 1963). A structure of formylbiliverdin has been suggested for

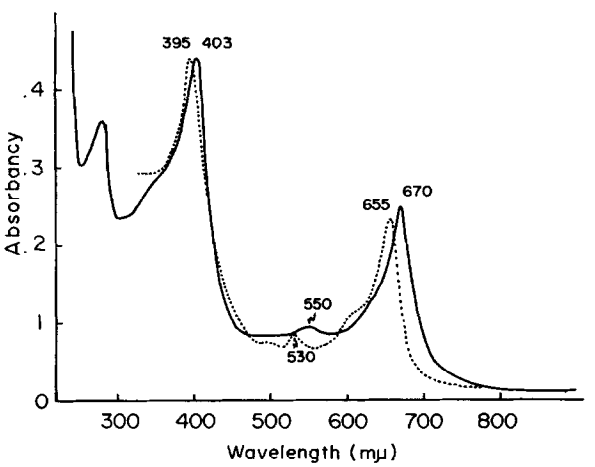

Fig. 2. Absorption spectra of purified P_{670} (solid line) and of pyridine complex of its chromophore (dotted line). P_{670} chromophore was isolated from the protein by HCl-acetone treatment and alkalline pyridine was added after acetone evaporation. A very similar spectrum was observed 20 minutes after alkalline pyridine was added to purified P_{670}.

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the compound which is a precursor of biliverdin (Nakajima, 1963; Nakajima and Gray, 1967).

In the experiment shown in Fig. 1, a further addition of IAA to the reaction solution changes the transformation product of HRP from P_{670} to

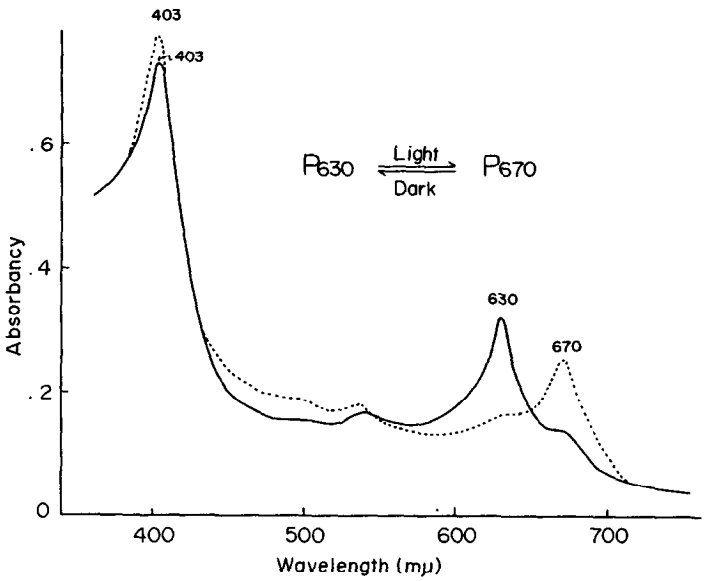

Fig. 3. Reversible conversion between P_{630} (solid line) and P_{670} (dotted line). P_{630} was prepared by 8 times repeated additions of 0.1 mM IAA into 15 μM HRP solution. 0.05 M acetate, pH 4.0, 20°. A small amount of P_{670} existed in this sample. A spectrum of P_{670} was taken during irradiating the sample with red light.

P_{630}. P_{630} can be seen only in the dark and is converted into P_{670} immediately after irradiation with red light as shown in Fig. 3.

$$P_{630} \underset{\text{dark}}{\overset{\text{red light}}{\rightleftharpoons}} P_{670}$$

The reverse reaction from P_{670} to P_{630} in the dark is relatively slow and its half-time is about 20 seconds at 18°. Although the light and dark reaction is repeated a number of times in the reaction solution as shown in Fig. 4, P_{630} which has been separated from the reaction solution with sephadex column in the dark is converted irreversibly into P_{670} upon irradiation with red light. Thus formed P_{670} does not return to P_{630} in the dark. No dark conversion can be observed from the purified P_{670} which is shown in Fig. 2. It is suggested from these results that certain factor is responsible to the reversible conversion between P_{630} and P_{670}.

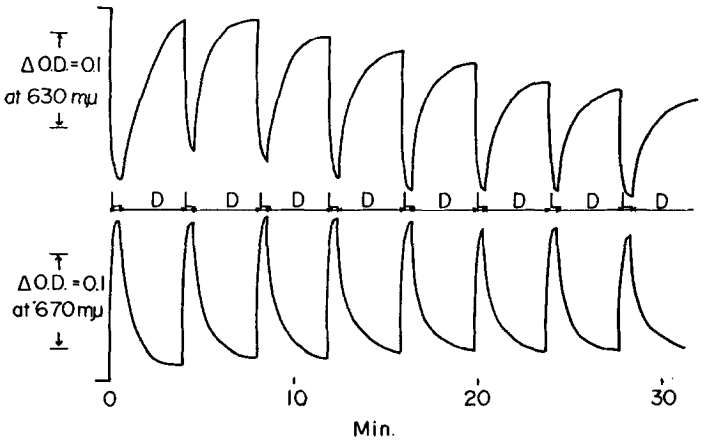

Fig. 4. Repeated light-dark conversion between P_{630} and P_{670}. Experimental conditions were same as that of Fig. 3 except HRP was 17 μM and temperature was 18°. The reaction was started from P_{630} by illumination with red light and light-dark phases are indicated in the centre line.

The factor may be closely related to the oxidation product of IAA.

Phytochrome isolated from various plant tissues is an interesting chromoprotein which shows the photoreversible changes and is now believed to act as an photoreceptor in the process of photoperiodism and photomorphogenesis (Siegelman and Hendricks, 1964; Hillman, 1967; Furuya, 1968). From the chromatographic and spectral properties Siegelman et al (1966) have suggested that the prosthetic group of phytochrome is bilitriene (Lemberg and Legge, 1949) closely similar to but distinct from the chromophore of C-phycocyanin and allophycocyanin. Our photoreversible compound is similar to phytochrome but obiously differs from it in the following points. 1. They have different absorption maxima. 2. Iron still exists in the prosthetic group of P_{670}. 3. Certain factors are in need for this photoreversibility. It is, however, very interesting to inquire into possible correlation between these two chromoproteins which is now in progress in our laboratory.

REFERENCES

Chance, B., Arch. Biochem., 21, 416 (1949).
Furuya, M., Progress in Phytochemistry, John Wiley and Sons, London, 1, 327 (1968).
Hillman, W. S., Ann. Rev. Plant Physiol., 18, 301 (1967).
Hinman, R. L. and Lang, J., Biochemistry, 4, 144 (1965).
Kajiro, K. and Kikuchi, G., J. Biochem., 38, 213 (1951).
Lemberg, R. and Legge, J. W., Hematin Compounds and Bile Pigments, Interscience, 1949, p 453.
Morita, Y., Kominato, Y. and Shimizu, K., Mem. Res. Inst. Food Sci., Kyoto Univ., 28, 1 (1967).
Nakajima, H., Takemura, T., Nakajima, O. and Yamaoka, K., J. Biol. Chem., 238, 3784 (1963).
Nakajima, H., J. Biol. Chem., 238, 3797 (1963).
Nakajima, O. and Gray, C. H., Biochem. J., 104, 20 (1967).
Ray, P. M., Ann. Rev. Plant Physiol, 9, 81 (1958).
Shannon, L. M., Kay, E. and Lew, J. Y., J. Biol. Chem., 241, 2166 (1966).
Siegelman, H. W. and Hendricks, S. B., Advan. Enzymol., 26, 1 (1964).
Siegelman, H. W., Turner, B. C. and Hendricks, S. B., Plant Physiol., 41, 1289 (1966).
Yamazaki, I. and Piette, L. H., Biochim. Biophys. Acta, 77, 47 (1963).
Yamazaki, I. and Souzu, H., Arch. Biochem. Biophys., 86, 294 (1960).
Yokota, K. and Yamazaki, I., Biochem. Biophys. Res. Comm., 18, 48 (1965).