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CYTOCHROME P-450 AND b_5 IN *CLAVICEPS PURPUREA*: INTERCONVERSION OF P-450 AND P-420

S. H. AMBIKE and R. M. BAXTER

Faculty of Pharmacy, University of Toronto, Toronto, Ontario, Canada

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Abstract—Cytochrome P-450 and b_5 have been shown to be present in *Claviceps purpurea*. The interconversion of cytochrome P-450 and cytochrome P-420 represents a unique property of the cytochrome P-450 which has been utilized in providing additional evidence for its presence in *C. purpurea*.

INTRODUCTION

IN AN earlier communication[1] the presence of cytochrome P-450 and b_5 in an alkaloid-producing strain of *Claviceps purpurea* was reported. It was observed that phenobarbitone (PB) and the polycyclic hydrocarbon 3-methylcholanthrene (3-MC) increased the level of cytochrome P-450 in this organism and that this increase was paralleled by an increase in alkaloid content. Ethionine and KCN which decreased alkaloid content caused a parallel decrease in cytochrome P-450.

The conversion of cytochrome P-450 to cytochrome P-420 is a unique property of the former and such a conversion represents a positive identification. Such a conversion can be readily carried out utilizing proteases, phospholipase, monohydric alcohols, amides, ketones, ureas, phenols, anilines, nitrites and sulfhydryl reagents and detergents[2] although the mechanism of conversion remains to be elucidated. Cytochrome P-420 gives the typical spectra of a b-type cytochrome. Ichikawa[2] has reported that after treatment with detergents or sulfhydryl reagents the cytochrome P-420 so formed may be converted back to P-450 by treatment with polyols or reduced glutathione. The present report provides data on the interconvertible property of the cytochrome P-450 reported[1] to be present in a mycelial pellet from *C. purpurea*.

RESULTS

The conversion of the CO-binding component (P-450) of the mycelial pellet, when converted into a solubilized form (P-420) by snake venom or sodium cholate, was accompanied by a well-defined alteration in its spectral properties.[3] The CO-difference spectrum of P-450 had a peak at 450 nm whereas that of P-420 was at 420 nm. From Fig. 1 it will be observed that the cytochrome P-450, as present in the steapsin-treated mycelial pellet, was converted to cytochrome P-420 after 60 min. Since cytochrome P-450 becomes labile to oxygen after steapsin treatment[4] it was necessary to carry out the steapsin digestion and subsequent treatment with heat-treated snake venom in an atmosphere of nitrogen.

[1] S. H. AMBIKE, R. M. BAXTER and N. D. ZAHID, *Phytochem.* 9, 1953 (1970).
[2] Y. ICHIKAWA and T. YAMARO, *Biochem. Biophys. Acta* 131, 490 (1967).
[3] T. OMURA and R. SATO, *J. Biol. Chem.* 239, 2370 (1964).
[4] T. OMURA and R. SATO, *Biochem. Biophys. Acta* 71, 224 (1963).

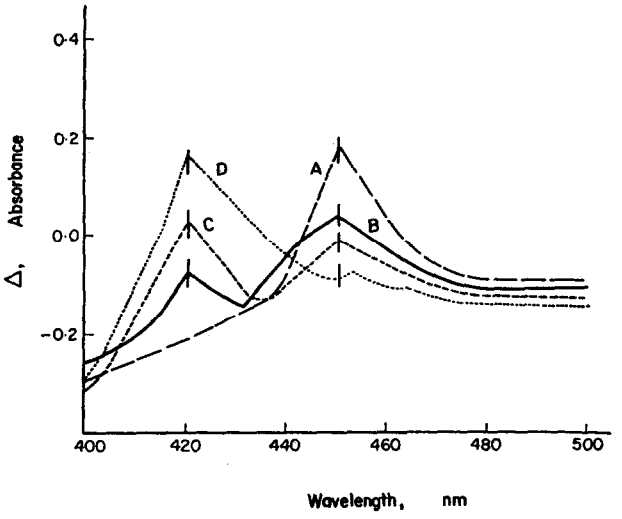

FIG. 1.

The CO-difference spectra of the cytochrome P-450 present in the mycelial pellet obtained from the control and phenobarbitone-treated *Claviceps purpurea* exhibited a difference spectra with an absorption maxima at 450 nm whereas that from 3-methylcholanthrene-treated *C. purpurea* exhibited an absorption maxima at 448 nm (Fig. 2). It will be observed also from Fig. 2 that after treatment with heat-treated snake venom all three exhibited the same absorption maxima at 420 nm. From Fig. 3 it will be observed that at a concentration of 4% (w/v) sodium cholate 96% of the cytochrome P-450 was converted to P-420.

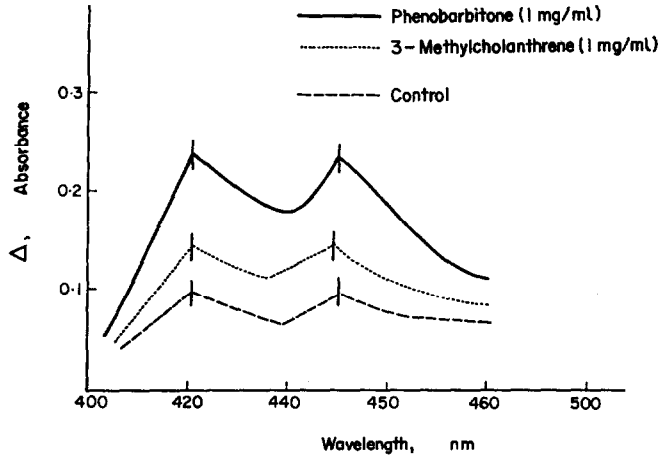

FIG. 2.

Figure 4 illustrates the reversibility of the conversion of the cytochrome P-420 to P-450 at various concentrations of glycerol. Thus the cytochrome P-450 present in the mycelial pellet was converted to P-420 on incubating the pellet with 4% (w/v) sodium cholate at 15° for 10 min. The cytochrome P-420 thus formed was reconverted back to P-450 to varying degrees

at various concentrations of glycerol. At a concentration of 10% (v/v) of glycerol the reconversion (P-420 to P-450) was 98% complete after 10 min at 15°.

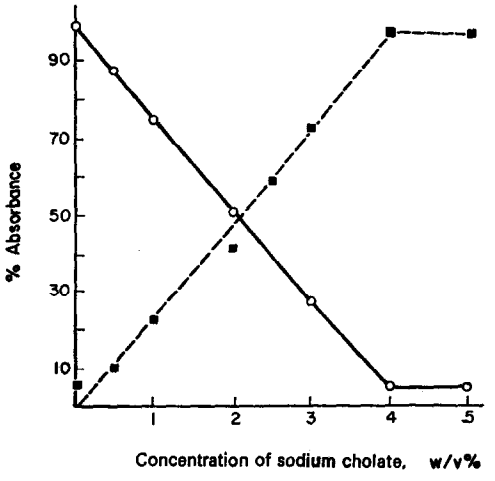

FIG. 3.

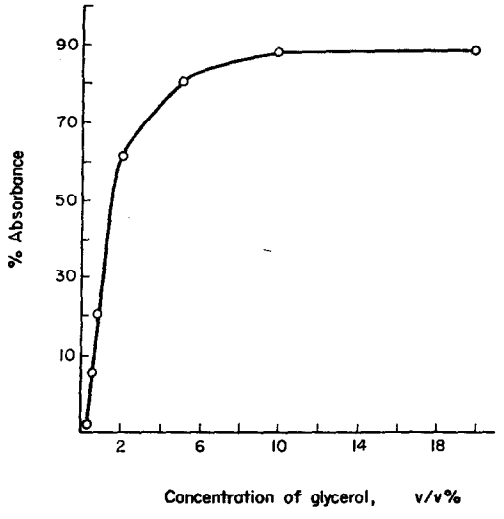

FIG. 4.

DISCUSSION

As indicated in an earlier communication[1] the mycelial pellet prepared from the fungus *Claviceps purpurea* contains a CO-binding hemoprotein. In liver cells the cytochrome P-450 is tightly bound to the microsomal membrane and undergoes a well-defined spectral change when treated with various reagents and is converted to a modified form (cytochrome P-420).[5] We have observed that when the mycelial pellet from the fungus *C. purpurea* was incubated

[5] Y. IMAI and R. SATO, *European J. Biochem.* **1**, 419 (1967).

anaerobically with snake venom or sodium cholate, the CO-binding component is converted to a soluble form exhibiting the spectral characteristics of cytochrome P-420. Digestion of the mycelial pellet with steapsin under suitable conditions liberated only cytochrome b_5 into solution leaving the CO-binding cytochrome attached to the residue. Heat-treated snake venom or sodium cholate solubilized the CO-binding protein from this residue. The spectra of the particulate and solubilized form of the CO-binding pigment from the mycelial pellet exhibited the characteristic spectra[3] of cytochrome P-450 and P-420 respectively. The 448 and 450 nm peaks may represent two spectrally different cytochromes or two interconvertible forms of the same cytochrome.[6] However, on solubilization, these were converted into spectrally indistinguishable forms exhibiting a single maximum absorption at 420 nm as has been reported for cytochromes from other sources.[6]

The selective solubilization of the cytochrome b_5 with steapsin,[4] although it leaves most of the CO-binding component in a bound form, results in the simultaneous conversion of cytochrome P-450 to cytochrome P-420. This conversion can be largely prevented by adding glycerol to the digestive mixture. The protective effect of glycerol in the cholate-induced conversion of cytochrome P-450 to cytochrome P-420 was first reported by Ichikawa, *et al.*[2] who assumed that polyols and GSH uncouple the detergents and sulfhydryl reagents from P-420 thus allowing reconversion to cytochrome P-450. The effect of glycerol in the reconversion of the CO-binding pigment of the mycelial pellet to a form exhibiting the spectral characteristics of cytochrome P-450 is reported here.

Thus the data presented here indicate the presence of a CO-binding component in the mycelial pellet of *C. purpurea* which exhibits spectral characteristics similar to that of the cytochrome P-450 found in certain animal and bacterial cells. The significance of the CO-binding component to alkaloid biosynthesis is currently being investigated and will constitute another report.

EXPERIMENTAL

Conversion of Cytochrome P-450 to P-420

The mycelial pellet was prepared from the *Claviceps purpurea* as previously reported.[1] The conversion of cytochrome P-450 to P-420 was effected as follows. The mycelial pellet was suspended in 0·1 M phosphate buffer (pH 7·5) and digested with 0·2% steapsin at 37° for 1 hr followed by centrifugation at 25,000 *g* for 2 hr. This removed most of the cytochrome b_5.

The pellet was suspended in 0·1 M tris buffer (pH 8·5) and incubated with heat-treated snake venom at a final concentration of 0·1% for 60 min at 4° in N_2. The incubation mixture was then centrifuged at 100,000 *g* for 60 min and the supernatant was utilized for determining the CO-difference spectrum by the method of Omura and Sato.[3] In another case the mycelial pellet, after steapsin treatment, was incubated at 15° for 10 min with various concentrations of sodium cholate.

The heat-treated snake venom was prepared by heating a 1% solution of the snake venom (Sigma Chemical) in 0·1 M tris buffer (pH 8·5) in a boiling-water bath for 5 min in order to inactivate most of the proteolytic activity of the crude venom. The precipitate was separated by centrifugation and discarded.

Conversion of Cytochrome P-420 to P-450

The reversibility of the conversion of P-420 to P-450 was studied using various concentrations of glycerol. The P-420 contained in the supernatant prepared as described above was treated with various concentrations of glycerol (0·5–20%, v/v) and incubated at 15° for 10 min. The cytochrome P-450 and P-420 were determined[3] by measuring the difference spectrum using a Beckman DU Spectrophotometer.

[6] A. P. ALVARES G. SCHILLING, W. LEVIN and R. KUNTZMAN, *J. Pharmacol. Exptl. Therap.* **163**, 417 (1968).