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Soluble, nitrate/nitrite-inducible cytochrome P-450 of the fungus, *Fusarium oxysporum*

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Both soluble and microsomal fractions of *Fusarium oxysporum* contain cytochrome P-450(P-450). We report here that the P-450 in the soluble fraction was induced only when nitrate or nitrite was added to the growth medium, whereas the microsomal P-450 was synthesized regardless of the medium compositions. The reduced-CO complex of the soluble P-450 exhibited an absorption spectrum that is different from that of the microsomal counterpart. These results indicate that the soluble P-450 is distinct from the microsomal species and suggest a novel function for the former P-450.

Cytochrome P-450; Nitrate/nitrite-mediated induction; Fatty acid hydroxylase; (*Fusarium oxysporum*)

1. INTRODUCTION

Following the discovery of the occurrence of cytochrome P-450 in the fungus *Fusarium oxysporum* [1], it was reported that this fungal P-450 could be recovered in both microsomal and soluble fractions and that its content in the soluble fraction was much higher than that in the microsomal fraction [2]. This observation was somewhat surprising because the existence of soluble P-450s was not known in eukaryotic organisms including yeasts [3,4] and fungi [5,6]. We now report that the soluble P-450 of *F. oxysporum* is a distinct species from the microsomal counterpart and that the former P-450 is specifically induced by nitrate or nitrite.

2. MATERIALS AND METHODS

F. oxysporum was cultivated as described [1,2]. The growth medium consisted of a basal medium containing inorganic salts, 0.2% soybean flour (or polypeptone), 3% soybean oil (or glycerol), and 0.2% sodium nitrate, sodium nitrite, or ammonium chloride. When 'low' aeration was desired, the fungus was grown in 3 l medium placed in a 5-l Erlenmeyer flask under agitation (on a rotary shaker) without a baffle. On the other hand, 'high' aeration was attained by growing the fungus in 1 l medium in the same flask with a baffle. The fungal cells were

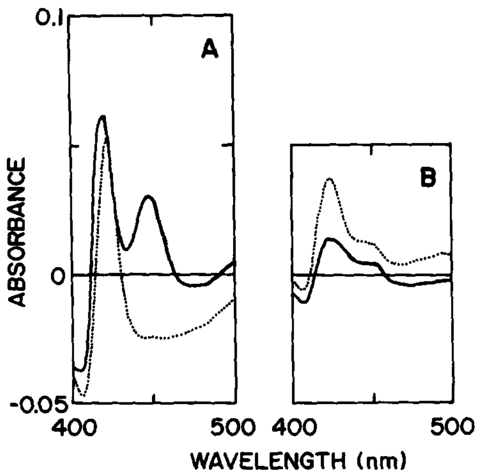

Fig.1. Carbon monoxide difference spectra of the soluble and microsome fractions of *F. oxysporum*. Each fraction was diluted 10-fold with 50 mM potassium phosphate buffer containing 20% glycerol, 2 mM mercaptoethanol, and 0.1 mM EDTA (pH 7.2). To avoid interference by cytochrome oxidase, the reference was not reduced with dithionite. (A) Soluble fraction, (B) microsome fraction. (——) Nitrate-grown cells; (···) ammonium-grown cells.

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Abbreviation: P-450, cytochrome P-450

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Table 1

Effects of culture conditions on production of cytochrome P-450 and fatty acid hydroxylase recovered in the soluble and microsome fractions of *F. oxysporum*

Medium		Aeration	Cell yield (wet[a] g/3 l medium)	Protein (mg/ml)		P-450 (nmol/mg protein)		Fatty acid hydroxylase (nmol product formed/min per mg protein)	
Carbon and nitrogen source	Inorganic nitrogen (0.2%)			Soluble	Microsome	Soluble	Microsome	Soluble	Microsome
Soybean oil and soybean flour	$NaNO_3$	low	55	9.76	5.88	0.288	0.075	0.092	0.15
	$NaNO_2$	low	27	11.1	5.95	0.685	0.103	0.028	0.032
	NH_4Cl	low	39	12.5	7.20	0.0035	0.11	0.15	0.25
	–[b]	low	47	2.52	4.72	–[c]	0.085	0.038	0.076
	$NaNO_3$	high	242	2.30	0.64	0.013	0.014	0.48	0.19
	$NaNO_2$	high	188	2.15	1.06	-	0.012		
Soybean oil and polypeptone	$NaNO_3$	low	36	11.6	5.15	0.565	0.158		
	$NaNO_2$	low	21	13.1	4.85	0.430	0.054		
	–	low	41	11.2	2.85	0.014	0.031		
Glycerol and soybean flour	$NaNO_3$	low	16	6.62	3.72	0.192	0.12	0.13	2.10
	NH_4Cl	low	35	7.96	8.20	0.014	0.040	0.064	0.89
Malt extract[e]	–	low	111	10.8	3.72	0.014	0.11	0.066[d]	

[a] Contained ~50% dry matter

[b] Not added

[c] Not detected

[d] Total of both fractions

[e] 1% malt extract, 0.4% yeast extract, and 1% glucose (pH 6)

disrupted, cell-free extracts were prepared, and the soluble and microsomal fractions were isolated as in [2]. Protein and P-450 were determined by the methods of Lowry et al. [7] and Omura and Sato [8], respectively. Fatty acid hydroxylase activities were assayed as described previously, using [1-^{14}C]lauric acid as substrate [2].

3. RESULTS AND DISCUSSION

A soybean flour-soybean oil medium containing sodium nitrate has been routinely used for cultivation of the P-450-producing fungus [1,2]. Here, it was unexpectedly found that no P-450 was recovered in the soluble fraction of the fungus grown in the medium in which nitrate was replaced by the same amount of ammonium chloride (fig.1A), although the P-450 content in the microsomal fraction was not greatly altered (fig.1B). This finding prompted us to study the effect of cultivation conditions on the induction of P-450 in more detail. As is evident from table 1, the presence of nitrate or nitrite in the medium was essential for the induction of P-450 in the soluble fraction. In contrast, the microsomal P-450 was synthesized regardless of the medium compositions, though its content varied depending on the media used. The use of 2% sodium glutamate or 2% polypeptone as carbon and nitrogen source also failed to induce the soluble P-450 in the absence of nitrate or nitrite (not shown). The induction was also affected by aeration. High aeration repressed the synthesis of P-450 in both fractions, in particular, that of P-450 in the soluble fraction even in the presence of nitrate or nitrite. Fatty acid hydroxylase activity, which had been shown to be a P-450-dependent reaction [2], could be detected in both fractions irrespective of the presence or absence of nitrate/nitrite. In most cases, however, the microsomal fraction exhibited higher specific activity than the soluble fraction. In particular, the glycerol medium was effective in inducing the high activity in the microsomal fraction. These results suggest that the activity is originally membrane-bound and solubilized in part during cell fractionation, as reported in [2]. It is evident from the results that the nitrate/nitrite-inducible P-450 is not responsible for the monooxygenase activity. Fig.2 shows the dependency of P-450 induction on nitrate concentration in the growth medium. As expected from the results in table 1, only the soluble P-450 exhibited a marked dose

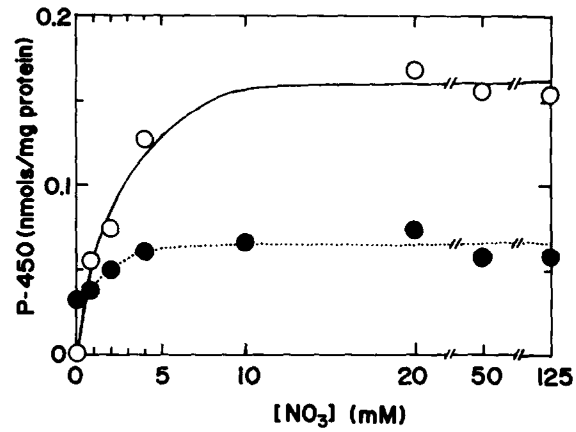

Fig.2. Nitrate-dose dependency of the induction of P-450 in *F. oxysporum*. Cells were cultivated in the presence of the indicated level of nitrate (soybean oil-polypeptone medium) under low aeration. P-450 content was determined with the soluble (○), and microsome (●) fractions.

dependency. A similar result was obtained with nitrite (not shown).

Fig.3 shows carbon monoxide-difference spectra

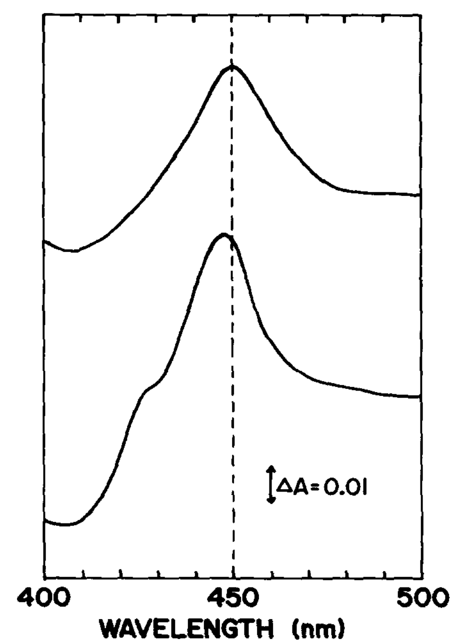

Fig.3. Carbon monoxide difference spectra of cytochrome P-450 in the microsome (upper) and soluble (lower) fractions of *F. oxysporum*. P-450 in the soluble fraction was purified as reported [1]. P-450 in the microsomal fraction was solubilized with sodium deoxycholate (0.15–0.8%), and then subjected to DEAE-cellulose column chromatography.

of the partially purified microsomal P-450 together with purified soluble P-450. The difference maximum is at 450 nm (microsome) and 448 nm (soluble), respectively.

Cytochromes P-450 recovered in both fractions revealed noticeable differences in conditions for induction, apparent localization, and spectral properties, showing that they are possibly different molecular species. The soluble P-450 exhibited several intriguing properties: induction by nitrate/nitrite, repression by aeration, apparent localization, and high production as compared with P-450s of other microbial origin. Induction of P-450 by nitrate has thus far not been established. The present results also suggested that the nitrate-inducible P-450 is intrinsically soluble though soluble P-450 has not been observed among eukaryotic cells. Judging from these unique properties, the soluble P-450 should possess some novel function as a P-450 which has not previously been demonstrated. On the other hand, the P-450 in the microsome fraction might be the 'traditional' monooxygenase(s) typically found in hepatic microsomes. One of the functions might be fatty acid hydroxylation.

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