SPECTRAL AND CATALYTIC PROPERTIES OF CYTOCHROME P-450 FROM A DIAZINON-RESISTANT HOUSEFLY STRAIN

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SUMMARY

Microsomes from the diazinon-resistant Rutgers strain of housefly contain amounts of cytochrome P-450 that are larger than those reported for rat liver, but the specific activity expressed as nmole of cytochrome P-450 per mg protein is much lower. The hemoprotein shows that spectral changes type I, II and IV are essentially in the low-spin form as judged by the n-octylamine and ethyl isocyanide difference spectra, and is unstable at pH below 6.5 and above 8.0. Cytochrome P-420 is also produced with time when CO-difference spectra are recorded. This is accelerated at pH above 8.0. The presence of contaminating amounts of cytochrome P-420, due to denaturation during spectral analysis or to the method used to isolate the microsomes, makes questionable the practice of characterizing the hemoprotein on the basis of the 455 nm peak in the ethyl isocyanide spectra, since a 434 nm peak is produced with concomitant decrease of the 455 nm peak. Microsomes hydroxylate naphthalene, aminopyrine and aniline, but the activity when expressed as nmole of product per nmole of cytochrome P-450 is the same or lower than that reported for other resistant housefly strains.

INTRODUCTION

Insect cytochrome P-450 associated to microsomal membranes has recently been extensively studied in several laboratories1−5. The hemoprotein is the terminal

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oxidase of a pyridine nucleotide-linked hydroxylating system which utilizes molecular oxygen, whereby one atom of oxygen is incorporated into the substrate while the other is reduced into water\(^6\). The parameters which affect the relationship between the levels of cytochrome P-450 and the hydroxylating activity of microsomal preparations have been clearly defined\(^3\). They include the type of substrate metabolized, the possibility of induction and the nature of the inducer, the affinity of the inducer and/or substrate for the hemoprotein active site, the rate of degradation of cytochrome P-450 after removal of the inducer, and the existence of different cytochrome P-450 species, appearing either through the action of an inducer or occurring naturally in different cell populations. Thus, cytochrome P-450 from resistant and susceptible housefly strains appears to be quantitatively different, as judged by spectral characteristics. The CO-difference spectra of microsomes from certain resistant strains show a maximum absorbance at 452 nm, while the diazinon-resistant Rutgers strain has a peak at 448 nm\(^8\). However, one susceptible strain also shows a peak at 452 nm\(^8\), while in another susceptible strain the peak is at 451 nm\(^2\). Cytochrome P-450 from the Fc strain maintained without insecticide pressure does not show type I spectral changes when oxidized, but only when the hemoprotein is reduced or previously incubated with ethyl isocyanide\(^4\). On the other hand, type I spectral changes are readily observed in the diazinon-resistant housefly strain\(^2\). However, some of the above reports should be considered with caution since the type of microsomal preparation used produce considerable denaturation of cytochrome P-450 into P-420\(^2\)\(^5\). As a result, ethyl isocyanide difference spectra yield peaks at 434 and 455 nm, instead of the usual 430 and 455 nm\(^4\). The 434 nm peak results from the interaction of cytochrome P-420 with ethyl isocyanide. Evidently, the characterization of hemoproteins from different sources based on the ethyl isocyanide-difference spectra alone, when cytochrome P-420 is present, is dubious, since the 434 nm peak will tend to mask the 455 nm one\(^2\).

Morello et al.\(^1\) have surmounted this problem through the use of a novel microsomal preparation from whole house flies. Under their conditions, microsomal fractions essentially free of cytochrome P-420 may be obtained\(^3\). Since the characteristics of the naphthalene- or phenobarbital-induced cytochrome P-450 from the Fc housefly strain appear to be close to those reported for the diazinon-resistant strain\(^2\)\(^4\), it was decided to examine the spectral and catalytic properties of the latter using the preparation of Morello et al.\(^1\). Firstly, the hemoproteins from both induced Fc and non-induced diazinon-resistant flies have the same maximum absorbance at 448 nm in the CO-difference spectrum; secondly, the levels of cytochrome P-450 in the diazinon-resistant strain are higher than those of the induced Fc strain\(^2\), but the latter's hemoprotein is unstable, which precludes its solubilization. If cytochrome P-450 from the diazinon-resistant strain could be shown to be the same as the Fc-induced hemoprotein, it would indicate that certain strains may have hemoprotein species that are only evident in other strains upon induction. Furthermore, the high levels of cytochrome P-450 in the diazinon-resistant strain would allow solubilization and purification studies even in the case of a certain degree of unstability. The spectral properties examined include the CO-, oxidized vs. reduced-, and n-octylamine-difference spectra, and spectral changes types I, II and IV (ref. 4).
The latter involves liganding with the heme of reduced cytochrome P-450 and is characterized by the appearance of two peaks in the Soret region at about 430 and 455 nm. This is seen with ethyl isocyanide and aniline. An estimation of the spin-state of the diazinon-resistant strain hemoprotein was of interest since it has been reported that it mainly is in the high-spin state. This would make it different from cytochrome P-450 from induced Fc flies, where preliminary experiments show that it is mainly in the low-spin state. The enzyme activity includes aromatic hydroxylation, as measured by the formation of 1-naphthol from naphthalene and p-aminophenol from aniline, and N-demethylation, as determined by the formation of formaldehyde from aminopyrine. In addition, the levels of NADPH-dependent cytochrome c reductase (EC 1.6.2.3) and of cytochrome bs have also been determined. The results obtained show that cytochrome P-450 from the diazinon-resistant strain has a maximum absorbance in the CO-difference spectrum at 448.5 nm, has typical spectral changes types I, II and IV (ref. 4), is highly unstable, and contrary to a previous report is essentially in the low-spin state.

METHODS AND MATERIALS

Insects
Non-sexed 5-day-old flies of the diazinon-resistant Rutgers strain were used throughout. The insects were maintained under constant malathion and diazinon pressure and reared in CSMA larvae medium obtained from Ralston Purina, St. Louis, Mo. The adults were fed ad libitum a mixture of non-fat powdered milk–sugar–powdered egg (6:6:1).

Preparation of microsomes
Microsomes were obtained from whole house flies by the “mortar” procedure of Morello et al. with the exception that the 0.1 M KH₂PO₄ buffer, pH 7.5 contained 10% (v/v) of glycerol. The microsomes were used immediately after preparation.

Spectral studies
All spectra were recorded at room temperature (22°C) in a Unicam SP-1800 spectrophotometer, using cuvettes of 1.0 ml capacity and 1.0 cm light path.

The CO- and ethyl isocyanide-difference spectra as well as the spectral changes types I and II were obtained as previously described. The n-octylamine difference spectra were obtained by the procedure of Jeftocoate et al.

NADPH-dependent cytochrome c reductase
The enzyme activity of microsomal preparations was determined by following the reduction of cytochrome c at 550 nm.

Hydroxylating activity
Ring hydroxylation of aniline and naphthalene and N-demethylation of amino-
pyrine were followed as described by Morello et al.\textsuperscript{1}, Capdevila et al.\textsuperscript{3,4} and Bleec-ker et al.\textsuperscript{10}.

Other analytical procedures have been previously reported\textsuperscript{1,3,4,10}.

Chemicals

Ethyl isocyanide was synthesized in the laboratory. 2-Diethylaminoethyl-diphenylpropyl acetate (SKF 525-A) was a gift from Smith, Kline and French, Philadelphia, Pa.; n-octylamine was obtained from Sigma Chemical Co., St. Louis, Mo. The rest of the chemicals used have been previously described\textsuperscript{1,3,4,10}.

All results reported here are the average of 4 to 7 experiments.

RESULTS

Characteristics of house fly microsomes

Microsomes isolated from the diazinon-resistant Rutgers strain contain about 3.3-fold more cytochrome P-450 than the Fc strain\textsuperscript{1,3}, but essentially the same amounts of cytochrome b\textsubscript{5} (Table I). The levels of NADPH-dependent cytochrome c reductase are about 2.5-fold higher than in the Fc strain\textsuperscript{3}. The specific activity of cytochrome P-450, expressed as nmole per mg protein, is also higher in the diazinon-resistant than in the Fc strain, 0.33 as compared to 0.265 nmole per mg protein\textsuperscript{1} This activity is in agreement with the values previously reported by others\textsuperscript{5}, which range from 0.29 to 0.38 nmole per mg protein when the data for microsomal protein of Folsom et al.\textsuperscript{11} or Perry et al.\textsuperscript{8} and an extinction coefficient of 91 cm\textsuperscript{-1} \( \cdot M^{-1} \) for the difference in absorbance between 450 and 490 nm in the CO-difference spectra\textsuperscript{1,3,4,12} are used.

<table>
<thead>
<tr>
<th>CHARACTERISTICS OF MICROSONES ISOLATED FROM THE DIAZINON-RESISTANT RUTGERS STRAIN OF HOUSEFLY</th>
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<td>The figures after the ± sign correspond to the standard error of the mean.</td>
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<tr>
<th>Total\textsuperscript{a}</th>
<th>per mg protein</th>
<th>per nmole cytochrome P-450</th>
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<tbody>
<tr>
<td>Protein, mg</td>
<td>62.5 ± 0.4</td>
<td>—</td>
</tr>
<tr>
<td>Cytochrome P-450 nmole\textsuperscript{b}</td>
<td>20.56 ± 2.1</td>
<td>0.33</td>
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<tr>
<td>Cytochrome b\textsubscript{5}, nmole\textsuperscript{c}</td>
<td>6.26 ± 0.5</td>
<td>0.1</td>
</tr>
<tr>
<td>NADPH-dependent cytochrome c reductase, μmole cytochrome c reduced per min</td>
<td>1.87 ± 0.15</td>
<td>0.03</td>
</tr>
<tr>
<td>Naphthalene hydroxylated, nmole per 15 min</td>
<td>998 ± 2.6</td>
<td>15.9</td>
</tr>
<tr>
<td>Aminopyrine N-demethylated, nmole per 15 min</td>
<td>387 ± 8.3</td>
<td>6.2</td>
</tr>
<tr>
<td>Aniline hydroxylated, nmole per 15 min</td>
<td>356 ± 3.0</td>
<td>5.7</td>
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\textsuperscript{a}Values expressed per 10 g flies. See METHODS AND MATERIALS for further details.

\textsuperscript{b}An extinction coefficient of 91 cm\textsuperscript{-1} \( \cdot M^{-1} \) for the difference in absorbance between 450 and 490 nm was used\textsuperscript{12}.

\textsuperscript{c}An extinction coefficient of 163 cm\textsuperscript{-1} \( \cdot M^{-1} \) for the difference in absorbance between 426 and 409 nm was used\textsuperscript{3,4}.
However, the total recovery of the hemoprotein is slightly less in the case of the Fc strain.

Microsomes from the diazinon-resistant strain hydroxylate 2.6-fold more naphthalene than the Fc strain, but the specific activity expressed as nmole of naphthalene hydroxylated per nmole of cytochrome P-450 is essentially the same in both strains. Although N-demethylation of aminopyrine is of the same magnitude in the diazinon-resistant and the Fc strains as expressed per 10 g of flies, the specific activity per nmole of cytochrome P-450 is about 2.8-fold less and about 0.5-fold less per mg protein in the case of the diazinon-resistant strain. It is interesting to note that a susceptible strain (NAIDM) shows negligible rates of naphthalene hydroxylation while another susceptible strain (CSMA) hydroxylates about 5.8 nmole of naphthalene per mg microsomal protein. Substantial amounts of p-aminophenol were formed from aniline by microsomes of the diazinon-resistant strain microsomes, while essentially no activity has been found in the Fc strain.

**CO- and oxidized vs. reduced-difference spectra**

Fig. 1 represents the CO-difference spectrum of cytochrome P-450 and the oxidized vs. reduced-difference spectrum of cytochrome b5 of microsomes from the diazinon-resistant strain. The absorbance maximum of cytochrome P-450 is at 448.5 ± 0.5 nm. The latter has been confirmed by the analysis of expanded spectra, not shown here. This maximum absorbance is essentially the same as reported by Perry et al. (448 nm) and Tate et al. (449 nm). As can be seen from Fig. 1, no
cytochrome P-420 is apparent, and essentially none appears when it is measured according to Omura and Sato\textsuperscript{12} by first bubbling CO into the sample cuvette and then adding Na\textsubscript{2}S\textsubscript{2}O\textsubscript{4} into both the sample and reference cuvettes. It should be pointed out that appreciable amounts of cytochrome P-420 were observed when microsomes of the diazinon-resistant strain were isolated by a different procedure\textsuperscript{2}, although no CO-difference spectra were reported. As will be shown later, a conversion of cytochrome P-450 into P-420 takes place with time, under the conditions used to obtain the CO-difference spectra of cytochrome P-450 which is contrary to what has been suggested by others\textsuperscript{3-5}, that is, that the "mortar" microsomal preparation used in this laboratory appears to be free of cytochrome P-420 because Na\textsubscript{2}S\textsubscript{2}O\textsubscript{4} denatures it under aerobic conditions. Omura and Sato\textsuperscript{12} have reported that cytochrome P-420 is labile to oxygen in the presence of dithionite. Furthermore, Imai and Sato\textsuperscript{15} have found that exposure of liver microsomes to neutral salts results in the conversion of cytochrome P-450 into P-420, a process that occurs more rapidly in the reduced state. Although cytochrome P-450 from the diazinon-resistant strain is unstable, no P-420 is formed in the CO-difference spectra assay at pH 7.5 during several minutes, a fact that may probably be due to the protective effect of glycerol\textsuperscript{12}.

The oxidized vs. the reduced-difference spectra of microsomal suspensions shows that cytochrome \textit{b} \textsubscript{4} has a maximum absorbance at 426 nm, as in the case of the Fc strain\textsuperscript{4}.

\textit{Spectral changes type I and II}

The addition of compounds such as aniline and other basic amines to mammalian and insect microsomes produces a spectral change characterized by the appearance of a trough at about 390 nm and a peak at about 431 nm, while addition of several ligands, such as substrates, inducers and xenobiotics, produces the appearance of a trough at about 390 nm and a peak at about 427 nm\textsuperscript{4,16}. Fig. 2 shows the spectral changes produced upon addition of aniline (spectral change type II) and SKF 525-A (spectral change type I) to microsomes from the diazinon-resistant strain. The position of the troughs and peaks for each spectral change is close to that reported for mammalian and diazinon-resistant housefly microsomes\textsuperscript{5} but differs from that reported for the Fc housefly strain microsomes\textsuperscript{4}. The type I spectral changes were readily obtained upon addition of SKF 525-A, or aminopyrine, but this is not the case in the Fc strain\textsuperscript{4}. It is interesting to note that type I spectral changes were not observed with a susceptible housefly strain\textsuperscript{5}. As with mammalian microsomes\textsuperscript{16}, the magnitude of the spectral change depended on the ligand concentration. When the reciprocal of the type I and II spectral changes was plotted against the reciprocal of ligand concentration (Fig. 3), the spectral dissociation constant ($K_s$), i.e. the concentration of ligand required for a half maximum spectral change, could be calculated. The values for aniline, aminopyrine and SKF 525-A were 0.213 M, 2.69 mM, and 1.40 mM, respectively. It should be pointed out that $K_s$ values for spectral changes type I are higher in microsomes of the Fc strain (3.90 mM) for SKF 525-A while $K_s$ values for aniline are lower (0.133 M)\textsuperscript{4}.

The trough of the type II spectral change in the diazinon-resistant strain is
asymmetrical (Fig. 2) with a small shoulder at about 410 nm. This is probably related to the observation that spectral changes type I are also present in type II (refs. 16, 17). As a matter of fact, by increasing the concentration of aniline, it is possible to obtain the “reverse type I” spectral change.

*Spectral changes type IV (ethyl isocyanide)*

The addition of ethyl isocyanide to chemically reduced microsomes resulted in the appearance of two peaks in the Soret region at 430 and 455 nm (Fig. 4A). At pH 7.0, cytochrome P-450 was not denatured, with essentially no cytochrome P-420 being present (Fig. 4B). At pH 8.0 (Fig. 4A), the 455 nm peak was slightly larger with a concomitant decrease in the 430 nm peak. The small amount of cytochrome P-420 that appears to contaminate the preparations at this pH (Fig. 4B) was not sufficient to displace the 430 nm peak. However, at pH 6.0 (Fig. 4A) the 430 nm peak disappeared and was replaced by a 433 nm peak, while the 455 nm peak was no longer apparent. The 433 nm peak can be correlated with the larger amounts of cytochrome P-420 present in the microsomal suspensions (Fig. 4B) with a concomitant decrease in cytochrome P-450. Cytochrome P-420 binds ethyl isocyanide producing a 434 nm peak, and the 433 nm peak observed at pH 6.0 (Fig. 4A) must correspond to a...

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Fig. 3. Plots of the reciprocal of spectral changes type I and II vs. the reciprocal of ligand concentration. Spectral changes as in Fig. 2. ●, SKF 525-A: $K_s = 1.40 \pm 0.41 \cdot 10^{-3} \text{ M}$; ○, aminopyrine: $K_s = 2.69 \pm 0.3 \cdot 10^{-3} \text{ M}$; △, aniline: $K_s = 0.213 \pm 0.055 \text{ M}$.

Fig. 4. Comparison between the ethyl isocyanide-difference spectra and the CO-difference spectra of microsomes from the diazinon-resistant strain at different pH. A, Ethyl isocyanide-difference spectra; B, CO-difference spectra. Spectra recorded within the first minute of ligand addition. Ethyl isocyanide, 0.2 M; microsomes, 4.2 mg protein per ml.
mixture of the 434 nm peak of cytochrome P-420 and the 430 nm peak of cytochrome P-450. At pH higher than 8.0, the amount of cytochrome P-450 rapidly decreased due to conversion into cytochrome P-420 (Fig. 4, insert). The pH at which the 430 and 455 nm peaks are of the same height is characteristic of a given cytochrome P-450 species. Thus, in the Fc housefly strain, this pH is 7.85, and is displaced to a higher pH when the insects are pretreated with phenobarbital or naphthalene*. However, in the case of the diazinon-resistant strain it was not possible to establish the pH at which both peaks are of the same height due to the instability of cytochrome P-450 at pH below 6.5 and above 8.0, and only an extrapolated value could be obtained from the relative height of the peaks at pH from 6.5 to 8.0 (Fig. 5). The extrapolated value was approximately 8.8.

**Stability of cytochrome P-450**

The changes in the absorbance at 450–490 nm (CO-difference spectra), 430–490 nm and 455–490 nm (ethyl isocyanide-difference spectra) at pH 9.0 and 7.5, with respect to time, are shown in Fig. 6. At pH 7.5 (Fig. 6B) cytochrome P-450 and the 430 and 455 nm peaks remain essentially unchanged up to 10 min. At pH 9.0 (Fig. 6A), the 430 nm peak is slowly displaced to the 434 nm region during the first 4 min and then rapidly afterwards. Parallel to the increase in the 434 nm peak, there is a decrease in the P-450 peak. The same occurs with the 455 nm peak. Evidently, the amount of the 434 nm peak is a function of the amount of cytochrome P-420 present. However, there is no strict correlation between the decrease in cytochrome P-450 and the decrease of the 455 nm peak. The $K_s$ values for the 430 and 455 nm peaks, determined at pH 7.5, were $3-4 \cdot 10^{-2} M$ and $8-9 \cdot 10^{-2} M$, respectively.

![Fig. 5. Ethyl isocyanide-difference spectra. Plot of the $\Delta E$ 430–490 nm per nmole of cytochrome P-450 vs. pH. Ethyl isocyanide, 0.2 $M$; protein, 4.63 mg per ml. Insert: Stability of cytochrome P-450 at different pH. Cytochrome P-450 concentrations obtained from CO-difference spectra using the extinction coefficient of 91 cm$^{-1}$ M$^{-1}$ (ref. 12).](image)

![Fig. 6. Plot of the absorbance at 450–490 nm, 455–490 nm and 434–490 nm vs. time at pH 9.0 (A) and 7.5 (B). Protein, 2.12 mg per ml; cytochrome P-450, 0.65 nmole per ml.](image)
**n-Octylamine-difference spectra**

*n*-Octylamine combines with two forms of oxidized cytochrome P-450 producing for the high-spin form a peak at 427 nm and a trough at 392 nm and for the low-spin form a peak at 432 nm and a double trough at 392 and 410 nm. Fig. 7 shows the type and magnitude of the spectral change caused by increasing concentrations of *n*-octylamine. The spectra obtained correspond to the low-spin form, obtained at even extremely low ligand concentrations. Saturation was reached at $3.5 \cdot 10^{-7} \text{ M}$ *n*-octylamine, and further increases in the concentration of the ligand did not reveal any high-spin form. When the ethyl isocyanide and *n*-octylamine-difference spectra were correlated, it was calculated that at pH 7.5 an ethyl isocyanide spectrum having a $\Delta E_{430}/\Delta E_{455} \text{ nm ratio of 5.8}$ should in theory correspond to an *n*-octylamine-difference spectrum having a $\Delta E_{410}/\Delta E_{392} \text{ nm ratio of 1.4}$. The experimental value obtained at a saturating concentration of *n*-octylamine was 1.16 (Fig. 7) which is within the experimental error of JEFCOATE et al.'s procedure.

**DISCUSSION**

Qualitative and quantitative differences between cytochrome P-450 from several housefly strains were first reported by PERRY et al. Differences in the ethyl isocyanide-difference spectra were later found by MATTHEWS AND CASIDA. New cytochrome P-450 species produced upon induction have recently been characterized. Our results confirm those of PERRY et al. and TATE et al. with respect to the absorbance maximum in the CO-difference spectrum of cytochrome P-450 from the diazinon-resistant strain (Fig. 1). They also show that this strain has the highest content of

![Fig. 7. n-Octylamine-difference spectra of microsomes from the diazinon-resistant housefly strain. Spectra obtained as described by JEFCOATE et al. Protein, 6.5 mg per ml; *n*-octylamine, $0.09 \cdot 10^{-7} \text{ M}$ (A), $0.6 \cdot 10^{-7} \text{ M}$ (B), $2.1 \cdot 10^{-7} \text{ M}$ (C), $3.5 \cdot 10^{-7} \text{ M}$ (D).]
cytochrome P-450 ever reported for housefly microsomes\textsuperscript{1–5,8}. As indicated in Table I, the contents of cytochrome P-450 per g fly is even higher than in rat liver preparations, and is rather close to the values given for phenobarbital-induced animals\textsuperscript{20}. However, its specific activity is about half that of mammalian hemoprotein and only 1.3-fold higher than the Fc strain microsomes\textsuperscript{1}. The contents of cytochrome \( b_5 \) is about half of that reported for rat liver microsomes when expressed per g fly, but the specific activity is much lower\textsuperscript{20}. The levels of NADPH-dependent cytochrome P-450 reductase are also higher than in the Fc strain, but the specific activity is essentially the same\textsuperscript{3}. However, it is approximately 10-fold lower than rat liver microsomes\textsuperscript{21}. In spite of the high levels of cytochrome P-450 in the diazinon-resistant strain, the production of \( p \)-aminophenol from aniline is lower than that of rat liver preparation\textsuperscript{11}. The cytochrome P-450 affinity for aniline in the diazinon strain is lower than that of the Fc strain\textsuperscript{4}, but the latter does not metabolize aniline at all\textsuperscript{15} (Figs. 2 and 3). Also, the total amounts of 1-naphthol produced from naphthalene are much higher in the diazinon strain than in the Fc strain\textsuperscript{1}, but when the specific activities are expressed as n mole of 1-naphthol produced per n mole of cytochrome P-450, they are essentially the same for both strains\textsuperscript{3}. In the case of aminopyrine, the specific activity is even lower for the diazinon-resistant strain\textsuperscript{3}. It would appear from these results that we still are not in a position to establish which is the rate-limiting step in microsomal hydroxylations, although the reduction of cytochrome P-450 seems to be of importance. The type of substrate metabolized is a factor, but the affinity of the hemoprotein for the substrate may play a secondary role. Whether allosteric or other factors play a part in the regulation of mixed-function oxidases in insects as well as in mammals\textsuperscript{4}, remains to be established.

Cytochrome P-450 from the diazinon-resistant strain is unstable at pH below 6.5 and above 8.0 and is rapidly denatured into cytochrome P-420 under the conditions used for determining CO-difference spectra (Figs. 4,5 and 6). This lability introduces some degree of uncertainty when ethyl isocyanide-difference spectra are obtained. A pH lower than 6.5 and higher than 8.0 the formation of cytochrome P-420 results in the appearance of a peak that is first at 433 nm and then shifts to 434 nm\textsuperscript{12} depending on the relative contribution of the 430 nm peak of cytochrome P-450 and the 434 nm peak of cytochrome P-420. This is paralleled by a decrease in the height of the 455 nm peak. Therefore, when contaminant cytochrome P-420 is present any quantitative expression of the ethyl isocyanide-difference spectra based only on the height of the 455 nm peak\textsuperscript{2–5} is highly questionable. The lability of the diazinon-resistant strain hemoprotein precluded an accurate estimation of the pH value at which the 430 and 455 nm peaks are of the same height, but an approximate pH of 8.8 (Fig. 5) was obtained by extrapolation, which is close to that reported for naphthalene- or phenobarbital-induced Fc houseflies\textsuperscript{4}.

A conflicting aspect is the \( n \)-octylamine-difference spectrum. The affinity of the diazinon-resistant strain cytochrome P-450 for \( n \)-octylamine is extremely high, the substrate concentration for a half-maximum change being around \( 1.75 \cdot 10^{-7} M \), which is four orders of magnitude lower than mammalian hemoprotein\textsuperscript{9} (Fig. 7). The
spectra obtained indicates that the hemoprotein is essentially in the low-spin form, which agrees well with the predominance of the 430 nm peak in the ethyl isocyanide spectra (Fig. 4). Higher concentrations of n-octylamine did not change the type of spectrum, as reported by Jeffcoat et al. However, other workers have described a typical high-spin n-octylamine-difference spectrum for the diazinon-resistant strain while a low-spin type of spectrum would correspond to susceptible housefly strains. The reasons for this discrepancy are not at all clear but the possibility exists that endogenous or exogenous substrates, which would tend to shift the low-spin form into a high-spin one, may be contaminating the microsomal preparations. The low-spin form of n-octylamine difference-spectrum found in this report correlates well with the spin characteristics observed in mammalian and in insect cytochrome P-450.

It has been reported that cytochromes P-450 and P-448 in the housefly may be regulated by genes carried on chromosomes II and V, respectively. Within the framework of our present knowledge it is difficult to ascertain if the characteristics of cytochrome P-450 from the diazinon-resistant and Fc strains are the manifestation of the above genetic traits or that other factors are involved. It has recently been found that it is not possible to establish a direct correlation between chromosome V, cytochrome P-448 and the epoxidase activity of microsomes from the Fc strain.

The possibility that cytochrome P-450 from the diazinon-resistant strain, which has a maximum absorbance in the CO-difference spectrum at shorter wavelengths than strains such as the Fc, may correspond to an induced species is intriguing. The high levels of the hemoprotein as well as the observation that induction shifts the Soret peak from 450 to 448 nm are indirect evidence supporting the above. However, the low specific activity of the diazinon-resistant strain hemoprotein towards naphthalene and other substrates are a basis for conjecture.

It is possible that proof of a possible identity of cytochrome P-450 from induced Fc and control diazinon-resistant flies may only be obtained when soluble and purified preparations are available. Work on the solubilization of insect microsomal mixed-function oxidases is currently being conducted in this laboratory.

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