Biochimica et Biophysica Acta, 314 (1973) 93–103

BBA 46575

SOME SPECTRAL PROPERTIES OF CYTOCHROME P-450 FROM MICROSOMES ISOLATED FROM CONTROL, PHENOBARBITAL- AND NAPHTHALENE-TREATED HOUSEFLIES*

JORGE CAPDEVILA[a], ALBERT S. PERRY[b], ANTONIO MORELLO[a,**] and MOISES AGOSIN[a,***]

[a]*Department of Zoology, University of Georgia, Athens, Ga. 30601 (U.S.A.) and* [b]*Center for Disease Control, U.S. Public Health Service, Savannah, Ga. 31402 (U.S.A.)*

(Received February 2nd, 1973)

SUMMARY

1. Phenobarbital and naphthalene induce a new species of cytochrome P-450 in houseflies which has a maximum absorbance in the off balance absolute spectrum at 446 nm.

2. Neither induced nor non-induced hemoprotein show type I spectral changes in the oxidized state but only when they are reduced by NADPH, $Na_2S_2O_4$ or when ethyl isocyanide is present in the reaction mixture. The affinity of the induced hemoprotein for type I and II substrates is greater than in the case of control cytochrome P-450.

3. As in mammalian systems, the type I spectral changes depend on the type I substrate concentration. However, control cytochrome P-450 shows small spectral changes at low substrate concentrations, and only at concentrations above 3 mM the spectral changes become large. On the contrary, appreciable type I spectral changes can be observed in the case of induced hemoprotein with substrate concentrations as low as 0.05 mM. This is particularly apparent in the case of induced hemoprotein previously incubated with ethyl isocyanide. Although the extinction coefficient for the Soret peaks produced by ethyl isocyanide appears to be the same for control and induced hemoprotein, the titration of spectral changes for the 455-nm peak is different in control and induced cytochrome P-450.

4. It is suggested that chemical changes involving the heme moiety of cytochrome P-450 bring about conformational changes in the protein moiety which facilitate the binding of type I ligands. Whether this phenomenon has an allosteric nature remains to be established.

Abbreviation: SKF 252-A, 2-diethyl aminoethyl diphenyl propyl acetate.

* In partial fulfillment of the requirements of the University of Georgia Graduate School for a Ph. D. degree (J.C.).

** Present address: Department of Chemistry and Biochemistry, University of Chile School of Medicine, Santiago, Chile.

*** To whom to address correspondence.

INTRODUCTION

Studies in several laboratories[1,2] suggest that cytochrome P-450 present in liver microsomes from 3-methylcholanthrene-treated rats is different from that found in non-induced or phenobarbital-treated animals[1]. The latter have an absorbance maximum in CO-difference spectrum at 450 nm, while the 3-methylcholanthrene-treated animals have a maximum absorbance at 448 nm, the so-called "cytochrome P-448". However, when an absolute CO-difference spectrum is recorded, cytochrome P-448 absorbs maximally at 446 nm[3,4]. This spectrally distinct hemoprotein has an altered catalytic activity[2,3,5]. Cytochromes P-450 and P-448 have been extensively characterized by ingenious spectral techniques, mainly those dealing with the absolute spectrum of the oxidized hemoprotein and the spectral changes induced by the addition of several ligands such as xenobiotics, inducers and inhibitors[6,7]. Four types of spectral changes have so far been described: (a) The type I spectral change, characterized by the appearance of a peak at approx. 390 nm and a trough at approx. 420 nm. Most substrates investigated produce a type I spectral change and this has been interpreted as a manifestation of the formation of a substrate–enzyme complex[6,8], (b) The type II spectral change is produced upon the addition of aniline and other basic amines and is characterized by the appearance of a trough at about 390 nm and a peak at about 420 nm[6,8]. (c) Phenacetin[8] and other compounds such as acetanilide produce the so-called modified type II[6,7], sometimes called "reversed type I" spectral change[8] which appears to be a reversal of the type I spectrum. (d) A fourth category involving liganding with the heme of reduced cytochrome P-450 is characterized by the appearance of two peaks in the Soret region at approx. 430 and 455 nm[9–11]. It is seen with ethyl isocyanide[9] and aniline[12]. The relative intensity of the two peaks is profoundly affected by changes in pH[13] and by the *in vivo* administration of polycyclic hydrocarbons[1]. More recent studies have shown that cytochromes P-450 and P-448 can be separated in a solubilized preparation as distinct species with different catalytic properties[14].

Treatment of houseflies with either naphthalene or phenobarbital results in the appearance of a CO-binding pigment which absorbs maximally at 448 nm, and which differs from the cytochrome P-450 of untreated houseflies in some catalytic activities and spectral properties[15]. In the present paper, we have extended our observations on the spectral properties of cytochrome P-450* isolated from naphthalene- and phenobarbital-treated insects, including the off balance spectrum[16] the characteristics of the type I and II spectral changes and the reduced ethyl isocyanide difference spectrum or type III spectral change. Our results indicate that the new hemoprotein species is a 446 type[15] of cytochrome P-450.

MATERIALS AND METHODS

The insect material, treatment with inducers and preparation of microsomal suspensions have been previously described[15,17].

* Since CO-difference spectra of cytochromes 450 nm, 448 nm and 446 nm are mentioned in the text, the above designation "cytochrome P-450" where applicable to the above spectra is for convenience only.

Chemicals

Ethyl isocyanide was synthesized in the laboratory. 2-Diethyl aminoethyl diphenyl propyl acetate (SKF 525-A) was a gift from Smith, Kline and French, Philadelphia, Pa. The rest of the chemicals used have been previously described[17].

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 reduced ethyl isocyanide difference spectrum was obtained as previously described[15]. Scanning was done over a wavelength from 390 to 500 nm.

The types I and II spectral changes were obtained by scanning over a wavelength from 370 to 470 nm. The ΔA for the type II spectral change was estimated from the difference in absorbance between the peak at 435 nm and the trough at approximately 395 nm. Control microsomes contained 7.5–9.0 mg of protein per ml and 0.80–1.04 nmoles of cytochrome P-450. Induced microsomes had a protein content of 8.3 to 10.2 mg/ml and 1.10 to 1.40 nmoles of cytochrome P-450. The ΔA for the type I spectral change was obtained from the difference between 410 and 428 nm. Control microsomes contained 8–9 mg of protein per ml and 1.02–1.10 nmoles of cytochrome P-450 per ml; phenobarbital- and naphthalene-treated microsomes contained 8.8–9.9 mg of protein per ml and 1.39–1.43 nmoles of cytochrome P-450 per ml. Microsomes suspended in 0.1 M potassium phosphate buffer, pH 7.5, were added to the sample and reference cuvettes and the following ligands for the type I spectral change were added to the sample cuvette at final concentrations varying between $4 \cdot 10^{-5}$ and $4 \cdot 10^{-2}$ M for aminopyrine; between $1 \cdot 10^{-5}$ and $1 \cdot 10^{-1}$ M for hexobarbital; between $1.5 \cdot 10^{-5}$ and $6 \cdot 10^{-3}$ M for SKF 525-A. Aniline was added at concentrations of $2 \cdot 10^{-2}$ to $1.5 \cdot 10^{-1}$ M to obtain type II spectral changes. The compounds were added consecutively to the sample cuvette, and the vehicle in which they were dissolved was added in the same amount to the reference cuvette.

The effect of type I ligands on type II spectral changes was investigated by adding type I substrates to both cuvettes prior to the addition of aniline to the sample cuvette.

The off-balance CO-difference spectrum was obtained as follows: the sample cuvette contained microsomes from induced insects (11 mg protein, 1.9 nmoles of cytochrome P-450, 1.45 nmoles of cytochrome b_5); the reference cuvette contained microsomes from control insects (10 mg protein, 1.2 nmoles of cytochrome P-450, 1.05 nmoles of cytochrome b_5). Solid $Na_2S_2O_4$ was added to both cuvettes, CO bubbled into both cuvettes for 30 s and the difference spectrum recorded between 390 and 500 nm.

Other spectral determinations and analytical procedures used have been previously described[15,17].

RESULTS

Off-balance CO-difference spectrum of cytochrome P-450

Fig. 1 illustrates the absorption spectrum obtained when microsomes derived from induced insects are placed in the sample cuvette, while the reference cuvette contains microsomes from the controls, the material from both cuvettes are reduced

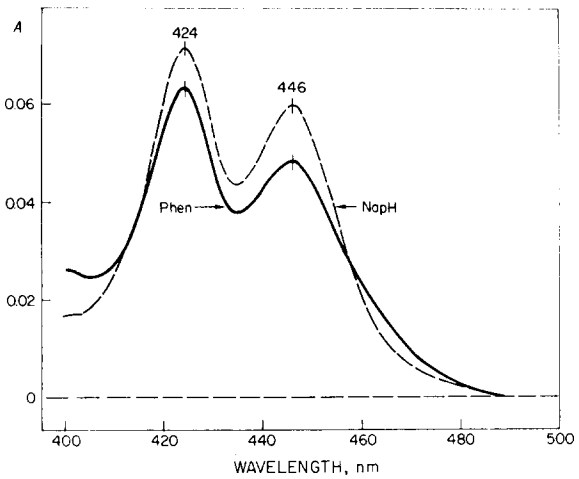


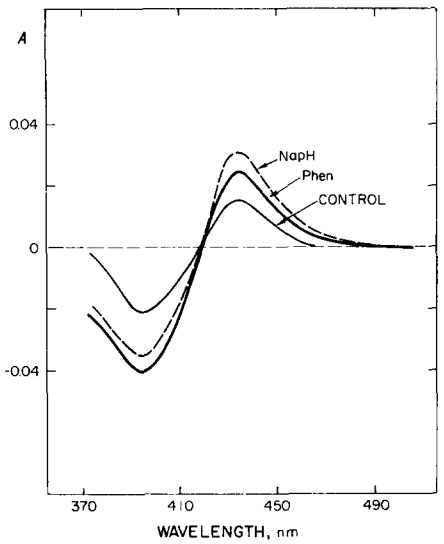
Fig. 1. Off-balance CO-difference spectra of cytochrome P-450 from phenobarbital (Phen) and naphthalene (NapH)-treated insects. See 'Materials and Methods'.

with $Na_2S_2O_4$ and then gassed with CO. The resultant spectral band represents the absorption spectrum of 0.61 nmole of cytochrome P-450 for phenobarbital- and 0.7 nmole of the same for naphthalene-treated insects. The spectral band has a maximum at 446 nm for both phenobarbital- and naphthalene-treated insects. The CO-difference spectrum of cytochrome P-450 isolated from microsomes from phenobarbital- or naphthalene-treated insects has a maximum at 448 nm[15] and this shift of 2 nm represents the combined contributions of induced and non-induced hemoprotein. Although it is difficult to establish the true maximum absorbance of the induced hemoprotein since this maximum is affected by the contribution of cytochrome b_5, it is suggestive that Hildebrandt and Estabrook[3] find that the absolute CO-difference spectrum of rabbit liver cytochrome P-450 induced by 3-methylcholanthrene has indeed a maximum absorbance at 446 nm. A balanced absolute CO-difference spectrum could not be done because cytochrome b_5 but not the protein are increased by phenobarbital or naphthalene.

It should be recalled that in mammals, the administration of polycyclic hydrocarbons results in a similar wavelength shift of the peak of maximum absorption for cytochrome P-450, but the administration of phenobarbital induces a cytochrome P-450 that does not differ from that of the control in its spectral characteristics[4,18].

Spectral changes type II

The addition of compounds such as aniline to mammalian liver microsomes results in the appearance of a trough at about 390 nm and a peak at 430 nm[6]. A similar phenomenon occurs in housefly microsomes (Fig. 2) but the peak is at approximately 435 nm and the trough at approximately 395 nm. As with mammalian microsomes[19] the magnitude of the spectral change depends on the concentration of the substrate added and, the type II spectral change in control microsomes is less pronounced than in induced ones at equimolecular aniline concentrations. When the reciprocal of the aniline induced type II spectral change is plotted against



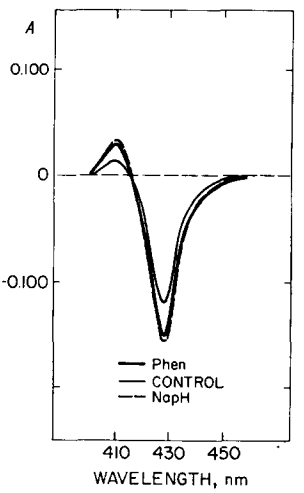


Fig. 2. Type II spectral changes induced in microsomes from control, naphthalene- and phenobarbital-treated insects. Aniline, 0.011 M. See Materials and Methods for further details.

Fig. 3. Type I-like spectral change of cytochrome P-450 from control and naphthalene- and phenobarbital-treated insects. Microsomes reduced with $1.3 \cdot 10^{-3}$ M NADPH. SKF 525-A added at a concentration of $4.6 \cdot 10^{-3}$ M for control microsomes and $3.5 \cdot 10^{-5}$ M for induced ones.

the reciprocal of substrate concentration and the spectral dissociation constant (K_s), *i.e.* the concentration of substrate required for a half maximum spectral change is calculated from a double reciprocal plot the K_s values for the induced cytochrome P-450 are the same for naphthalene and phenobarbital (0.064 M) but are lower than the control values (0.133 M).

Spectral changes type I

When substrates such as SKF 525-A, hexobarbital, phenobarbital or aminopyrine, all type I substrates, are added to housefly microsomal suspensions, no spectral changes are observed even at high substrate concentration. However, when the microsomal suspensions are reduced with either NADPH or $Na_2S_2O_4$, a type I-like spectral change is consistently observed, but with a small peak at 410 nm and a trough at 428 nm (Fig. 3). It should be pointed out that mammalian microsomes show a type I spectral change upon addition of several substrates, with a peak at 385 nm; the latter is increased and displaced to 389 nm when reduction is carried out with NADPH[20]. Similarly, the trough is more pronounced with a band at 423 nm, instead of 420 nm showed by non-reduced microsomes. At a given substrate concentration, the changes were more pronounced in the case of induced cytochrome P-450. It should be pointed out that variations in the height of the peak at 410 nm were much less pronounced than the variations in the size of the trough at 428 nm.

The type of curve obtained precluded the calculation of a true K_s value, since a plot of the reciprocal of the spectral change *versus* the reciprocal of substrate concentration does not show a straight line (Fig. 4). Therefore, only approximate

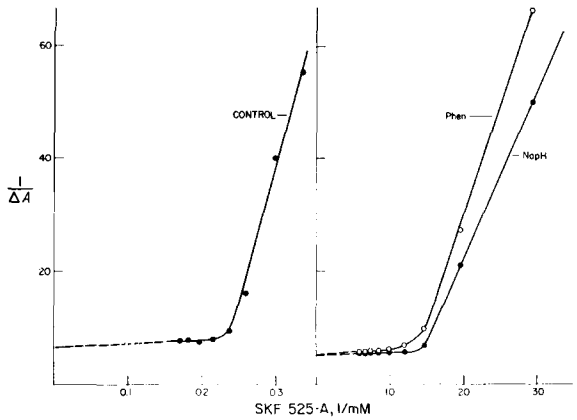


Fig. 4. Plot of the reciprocal of the type I-like spectral change *versus* the reciprocal of SKF 525-A concentration in NADPH-reduced cytochrome P-450 from control, naphthalene- and phenobarbital-treated insects. The dotted line is obtained by extrapolation.

K_s values are given as obtained by dividing the maximal spectral change by 2 (ref. 21). The approximate K_s values for control, naphthalene- and phenobarbital-treated insect microsomes are given in the insert of Figs 5A and 5B. It is interesting to note that the induced hemoprotein has K_s values that at least are 60-fold lower than that of the control.

It is evident from Fig. 5A that small changes are obtained in the case of control cytochrome P-450 at low substrate concentrations which only increase at high substrate concentrations. However, in the case of induced cytochrome P-450 (Fig. 5B) large type I-like changes were obtained at low SKF 525-A concentrations. Similar results are obtained with other type I substrates such as aminopyrine and hexobarbital. Type I-like spectral changes can also be obtained when the microsomes

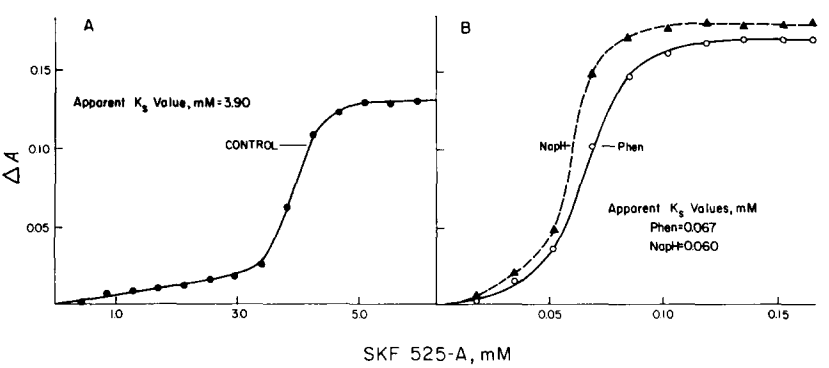


Fig. 5. Effect of changes in the concentration of SFK 525-A on the magnitude of the type I-like spectral change of NADPH-reduced microsomes. NADPH added at the final concentration of $1.3 \cdot 10^{-3}$ M. (A) Control microsomes: 9.6 mg of protein and 1.02 nmoles of cytochrome P-450 per ml. (B) Naphthalene-induced microsomes: 10 mg of protein and 1.48 nmoles of cytochrome P-450 per ml; phenobarbital-induced microsomes: 10.8 mg of protein and 1.40 nmoles of cytochrome P-450 per ml. "Approximate" K_s calculated from the concentration of substrate producing half of the maximal spectral change.

are first allowed to react with ethyl isocyanide in the absence of NADPH and $Na_2S_2O_4$ (Figs 6A and 6B). Under these conditions, changes in the concentration of a type I substrate such as SKF 525-A, essentially produces the same spectral changes as those shown for microsomes reduced with either NADPH or $Na_2S_2O_4$. Although the approximate K_s values are similar for control microsomes reduced either with NADPH or $Na_2S_2O_4$ (Fig. 6A) the approximate K_s values for SKF 525-A with induced microsomes are decreased (Fig. 6B). The approximate K_s values were obtained as indicated above.

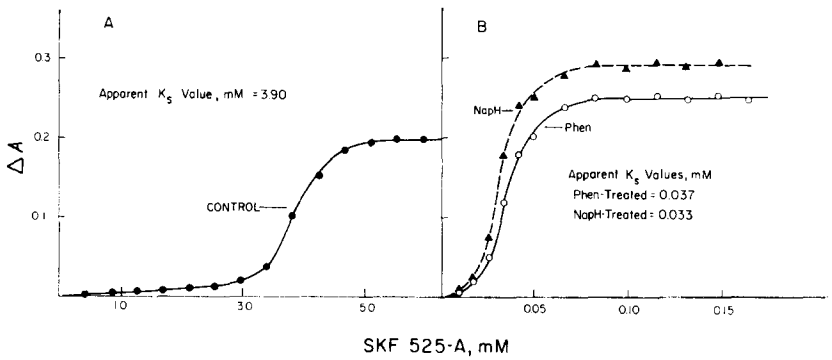


Fig. 6. Effect of changes in the concentrations of SKF 525-A on the magnitude of the type I-like spectral change of oxidized microsomes to which ethyl isocyanide was added at a final concentration of 0.1 M. Concentration of protein and cytochrome P-450 as in Figs. 5A and 5B Approximate K_s values determined as in Fig. 5.

The possibility that type I substrates indeed bind to oxidized cytochrome P-450 but without apparent spectral changes is supported by experiments (unpublished) which indicate that the addition of SKF 525-A to a microsomal suspension in the presence of aniline produces approximately a 20% increase in the magnitude of the type II spectral change induced by the latter, an observation already reported by Schenkman[19].

Ethyl isocyanide difference spectrum

The addition of ethyl isocyanide to housefly microsomes results in the appearance of two peaks in the Soret region at 430 and 455 nm, similar to the peaks of mammalian cytochrome P-450[9,15]. The pH at which the 430- and 455-nm peaks are of the same height differs for control and induced cytochrome P-450[15].

However, the results of Fig. 7 suggest that the extinction coefficient of the 430- and 455-nm peaks is the same for both control and induced hemoproteins. When the ratios $\Delta A_{430-490\ nm}/\Delta A_{450-490\ nm}$, as well as $\Delta A_{455-490\ nm}/\Delta A_{450-490\ nm}$ are plotted against pH, the resulting curves essentially have the same intercept in the y axis in control and induced hemoproteins. The $\Delta A_{450-490\ nm}$ values were obtained from CO-difference spectra of the same microsomal suspensions utilized to obtain the ethyl isocyanide difference spectra. Similar results have been obtained with phenobarbital and 3-methylcholanthrene in a mammalian system[21]. Whether these observations indicate that control and induced hemoprotein have the same extinction coefficient in the CO-difference spectrum is difficult to ascertain at present.

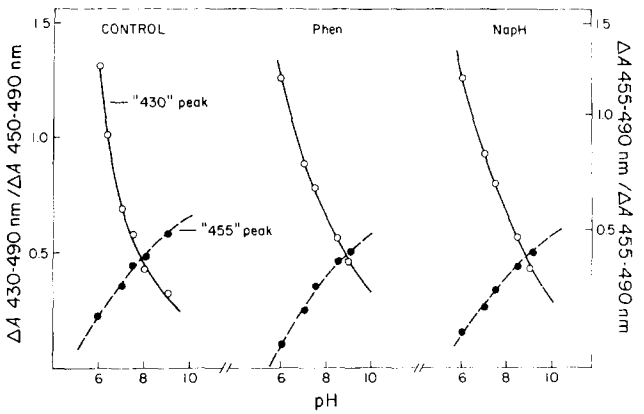


Fig. 7. Ethyl isocyanide difference spectra. The ratios $\Delta A_{430-490\ nm}/\Delta A_{450-490\ nm}$ and $\Delta A_{455-490\ nm}/\Delta A_{450-490\ nm}$ are plotted against pH. The values 450-490 nm were obtained from a CO-difference spectrum.

Nebert[22] has reported that cytochrome P-450 from control and induced cell cultures have the same extinction coefficient, but others claim they differ[23].

In order to determine the effect of ethyl isocyanide concentration on the size of the 430- and 455-nm peaks at a fixed pH of 7.5, the ratios $\Delta A_{455-490\ nm}/\Delta A_{430-490\ nm}$ were obtained at different ethyl isocyanide concentrations. These ratios were then plotted against the logarithm of the ethyl isocyanide concentration (Fig. 8). Both the 430- and 455-nm peaks increase in magnitude at the same rate with increasing amounts of ethyl isocyanide in control microsomes. As a result, a straight line is obtained. On the other hand, only the 430-nm peak increases at a constant rate with increasing concentrations of ethyl isocyanide in the case of induced microsomes, but the 455-nm peak increases to larger values when the concentration of ethyl isocyanide is above 7 mM. This is further indication that the induced hemoprotein is different from the control one. The values for the maximum height of each Soret peak were obtained from double reciprocal plots

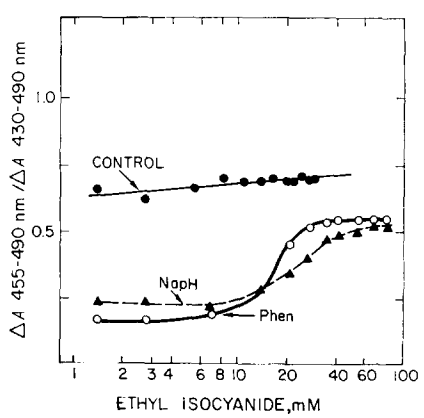


Fig. 8. Plot of the height of the 430- and 455-nm peaks induced by ethyl isocyanide *versus* the logarithm of ethyl isocyanide concentration.

when the concentration of ethyl isocyanide was varied from 1.35 to 80 mM. The approximate K_s values for each peak were obtained from the concentration of ethyl isocyanide required for a 50% change. The K_s for control microsomes was between 5 and 6 mM for both Soret peaks. The K_s for the 430-nm peak for naphthalene- and phenobarbital-treated insect microsomes was approximately 10 mM. However, the K_s value for the 455-nm peak for naphthalene–hemoprotein was about 20 mM and for phenobarbital–hemoprotein between 17 and 18 mM. It should be pointed out that for this type of experiments, repeated addition of increasing concentrations of ethyl isocyanide results in a conversion of the 430- to the 455-nm peak with time. For this reason, each point in Fig. 8 corresponds to a given concentration of ethyl isocyanide added to a fresh microsomal suspension.

DISCUSSION

The evidence presented here together with the data previously reported[15] indicate that treatment of insects with naphthalene or phenobarbital gives rise to the appearance of a cytochrome P-450 species that is different from that found in untreated insects. The new species as well as the normally present also differ in some characteristics from the CO-binding hemoprotein described for other organisms. The off-balance CO-difference spectrum (Fig. 1) suggests that the new species of cytochrome P-450 has a maximum absorbance at 446 nm, similar to the liver hemoprotein induced by 3-methylcholanthrene and benzopyrene[3]. Therefore, phenobarbital acts in insects as polycyclic hydrocarbons do in mammals. It could be argued that the shift in absorption is due to the presence of residual phenobarbital or naphthalene. However, naphthalene is metabolized very rapidly by insect microsomes[17]. Although residual phenobarbital could exist[15], addition of 2 mM phenobarbital to control microsomal suspensions does not shift the maximum absorption of cytochrome P-450 in the CO-difference spectrum.

The reduced, ethyl isocyanide difference spectrum also indicates that a higher pH is required for equalization of the two Soret peaks in the new species[15]. Although the results of Fig. 7 suggest that the extinction coefficients of the Soret peaks produced by the addition of ethyl isocyanide is the same for control and induced hemoprotein, the affinity of each peak for ethyl isocyanide is lower for induced cytochrome P-450. However, the affinity of both control and induced housefly cytochrome P-450 for ethyl isocyanide is much lower than that reported for mammalian cytochrome P-450[12].

The type II spectral changes in insect cytochrome P-450 are similar to those described for mammals[19]. Induced cytochrome P-450 shows greater spectral changes than non-induced one at equal substrate concentration. In addition, the magnitude of the K_s value for aniline is lower for induced hemoprotein. The K_s values for the spectral change type II are extremely high as compared to mammalian systems[19]. This lower affinity for aniline may be responsible for the low metabolic rate of housefly microsomes towards aniline (Morello, A. and Agosin, M., unpublished results).

The observation that neither control nor induced oxidized cytochrome P-450 show type I spectral changes is not without precedent. Cortisol and cortisol 21 sodium succinate produce a type I spectral change with adrenal microsomes, but with liver microsomes, these changes are weak or non existent[24]. On the other hand, aminopyrine and hexobarbital, that produce a marked type I spectral change

in liver microsomes, do not show a change in adrenal microsomes. Remmer *et al.*[25], Schenkman[19], and Schenkman *et al.*[6,7] have indicated that type I changes may be weak or absent because residual substrate is already interacting with binding site I. However, housefly microsomes isolated from control insects also show the inability to produce type I spectral changes. The lack of spectral manifestations upon the addition of type I substrates to insect microsomes does not reflect an absence of interaction of the substrate with binding site I, since spectral changes type II induced by aniline are magnified by the addition of type I substrates. Philpot and Hodgson[26] were unable to show type I spectral changes in microsomes isolated from a susceptible housefly strain. Furthermore, only the addition of benzphetamine to microsomes derived from a resistant strain produced a type I spectral change while other type I substrates were ineffective. Type I-like spectral changes can be obtained when the microsomes are previously reduced either with NADPH or $Na_2S_2O_4$ or when the microsomes in the oxidized state are incubated with ethyl isocyanide prior to the addition of the type I substrate (Figs 4, 5A, 5B, 6A and 6B). Once again, the approximate K_s values for the induced hemoprotein are lower than the control. It would appear that when the hemoprotein iron atom is modified by reduction with either NADPH or $Na_2S_2O_4$, conformational changes of the protein moiety are brought about. The ethyl isocyanide supported conformational changes appear to be of a more profound nature, since the type I spectral changes are much more pronounced than when the hemoprotein is reduced. This is particularly evident in the case of induced hemoprotein (Figs 5 and 6). Since the Soret peaks induced by ethyl isocyanide are pH dependent (Fig. 7), it would appear that the effects of this abduct is mediated through protein changes, further supporting that the conformational changes produced by ethyl isocyanide in the case of housefly cytochrome P-450 involve the protein moiety which in turn change the environment of binding site I. However, changes in ionization of ethyl isocyanide may also participate in this phenomenon. Summing up, it would seem that any chemical modification of cytochrome P-450 involving the heme moiety will result in conformational changes of the protein moiety with the appearance of type I-like spectral changes. This is not the case of mammalian cytochrome P-450, where type I spectral changes are observed upon addition of type I ligands without previous reduction or treatment of the microsomes. The observation that changes type I are much more pronounced in induced hemoprotein (Figs 5 and 6) as well as the behavior of the ethyl isocyanide-produced 455-nm peak at different ligand concentrations in control and induced insects (Fig. 8) further supports our contention that induction gives rise to a new cytochrome P-450.

ACKNOWLEDGMENT

This work was supported in part by U.S. Public Health Service Research Grant AI-09902 (to M.A.).

REFERENCES

1 Sladek, N. E. and Mannering, G. J. (1966) *Biochem. Biophys. Res. Commun.* 24, 668–674
2 Alvares, A. P., Schilling, G., Levin, W. and Kuntzman, R. (1968) *J. Pharmacol. Exp. Ther.* 163, 417–424

3 Hildebrandt, A. G. and Estabrook, R. W. (1969) in *Microsomes and Drug Oxidations* (Gillette, J. R., Conney, A. H., Cosmides, G. T., Estabrook, R. W., Fouts, J. R. and Mannering, G. J., eds), pp. 331–343, Academic Press, New York
4 Alvares, A. P., Schilling, G., Levin, W. and Kuntzman, R. (1968) *Biochem. Biophys. Res. Commun.* 29, 521–526
5 Kuntzman, R., Lu, A. Y. H., West, S., Jacobson, M. and Conney, A. H. (1971) *Chem.-Biol. Interactions* 3, 287–288
6 Schenkman, J. B., Remmer, H. and Estabrook, R. W. (1967) *Mol. Pharmacol.* 3, 113–123
7 Schenkman, J. B., Greim, H., Zange, M. and Remmer, H. (1969) *Biochim. Biophys. Acta* 171, 23–31
8 Schenkman, J. B., Cinti, D. L., Orrenius, S., Molduns, P. and Kaschnitz, R. (1972) *Biochemistry* 11, 4243–4252
9 Omura, T. and Sato, R. (1964) *J. Biol. Chem.* 239, 2379–2385
10 Philpot, R. M. and Hodgson, E. (1972) *Chem. Biol. Interactions* 4, 185–194
11 Philpot, R. M. and Hodgson, E. (1971) *Life Sci.* 10, 503–512
12 Imai, Y. and Sato, R. (1967) *J. Biochem. Tokyo* 62, 464–473
13 Imai, Y. and Sato, R. (1966) *Biochem. Biophys. Res. Commun.* 23, 5–11
14 Lu, A. Y. H., Kuntzman, R., West, S. and Conney, A. H. (1971) *Biochem. Biophys. Res. Commun.* 42, 1200–1206
15 Capdevila, J., Perry, A. S., Morello, A. and Agosin, M. (1973) *Biochemistry* 12, 1445–1451
16 Remmer, H., Estabrook, R. W., Schenkman, J. B. and Greim, H. (1968) *Naunyn-Schmiedeberg's Arch. Pharmakol. Exp. Pathol.* 259, 98–116
17 Morello, A., Bleeker, W. and Agosin, M. (1971) *Biochem. J.* 124, 199–205
18 Hildebrandt, A. G., Remmer, H. and Estabrook, R. W. (1968) *Biochem. Biophys. Res. Commun.* 30, 607–612
19 Schenkman, J. B. (1970) *Biochemistry* 9, 2087–2091
20 Remmer, H., Schenkman, J., Estabrook, R. W., Sasame, H., Gillette, J., Narasimhulu, S., Cooper, D. Y. and Rosenthal, O. (1966) *Mol. Pharmacol.* 2, 187–190
21 Imai, Y. and Siekeritz, P. (1971) *Arch. Biochem. Biophys.* 144, 143–159
22 Nebert, D. W. (1970) *J. Biol. Chem.* 245, 519–527
23 Mannering, G. J. (1971) *Metabolism* 20, 228–248
24 Orrenius, S., Kupfer, D. and Ernster, L. (1970) *FEBS Lett.* 6, 249–252
25 Remmer, H., Schenkman, J. B. and Greim, H. (1969) in *Microsomes and Drug Oxidations* (Gillette, J. R., Conney, A. H., Cosmides, G. J., Estabrook, R. W., Fouts, J. R. and Mannering, G. J., eds), pp. 371–386, Academic Press, New York
26 Philpot, R. M. and Hodgson, E. (1972) *Chem.-Biol. Interactions* 4, 399–408