THE SOLUBILIZATION AND SEPARATION OF TWO FORMS OF MICROSOMAL CYTOCHROME P-450 FROM THE HOUSE FLY, MUSCA DOMESTICA L.

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Summary: Microsomes prepared from house fly abdomens were solubilized with sodium deoxycholate and resolved on a DEAE cellulose column into two fractions containing cytochrome P-450. On the basis of spectral characteristics the cytochrome in a fraction eluting with 0.3M KCl is different from that eluting with 0.5M KCl. The two forms of P-450 were found in both susceptible and resistant strains of house flies. The P-450 in the 0.5M KCl eluting fraction appears to be more unstable than that in the 0.3M fraction. These results are in disagreement with those of other workers who have concluded that only one of two possible forms of cytochrome P-450 is present in a given strain of the house fly.

Although cytochrome P-450 plays the same important role in the insect's metabolism of both foreign (1) and endogenous (2) compounds as it does in higher animals, its characterization in insects has not kept pace with that occurring in other areas. Instead, there appears to be considerable confusion about insect cytochrome P-450, especially in the case of the house fly which has been studied most extensively. The current theory is that two forms of P-450 are present in house flies, one which exhibits a carbon monoxide difference spectrum with λ_{max} at 448-449 nm and is found in insecticide resistant strains, and the other with CO·P-450 of λ_{max} 451-452 nm which is present in susceptible strains (3). There are exceptions to this, however, with one report showing the 452 nm "susceptible" form of P-450 in several resistant strains (4) and another concluding that one form of cytochrome P-450 with CO·P-450 λ_{max} about 450 nm is normal to the Fc (resistant) strain while a 446 nm type occurs when that strain is induced with phenobarbital or naphthalene (5).

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The problems encountered in studying cytochrome P-450 in insects have been discussed by Hodgson et al. (3) and by Capdevila et al. (6). Both authors agree that further progress in solving these problems will require the development of methods for solubilizing and resolving insect microsomes into their active components. We now report some progress in this direction and we conclude that at least two forms of cytochrome P-450 are present in both R and S house flies.

Methods: Two insecticide susceptible fly strains, NAIDM and SRS, and two resistant strains, Isolan-B and Fc, were used in these experiments. The enzymatic and toxicological parameters for these strains have been published (7). Several thousand female flies were frozen, 20 g of abdomen removed and homogenized, and microsomes prepared as previously described (8). The microsomes were resuspended in 0.07 M Tris-HCl buffer pH 7.5, containing 0.025 M sucrose, 1 mM dithiothreitol (DTT), and 30% glycerol, and homogenized in a hand operated tissue grinder. The suspension was diluted to 1 ml of buffer/800 mg of abdomen, and stored overnight at -10°C.

The thawed microsome suspension was treated at room temperature with 2 mg sodium deoxycholate (DOC)/ml with occasional further homogenization for 30 min. The suspension was then introduced into a DEAE cellulose column followed by two 10 ml rinses with eluting buffer and 1 column volume (50-60 ml) of 0.1M KCl buffer. Step-wise elution was carried out with increasing concentrations of KCl in the eluting buffer which consisted of 0.1M Tris-HCl, pH 7.6, 1mM DTT, 20% glycerol, and 0.05% DOC. The column flow rate was maintained at approximately 1 ml/min, while 10 ml fractions were collected.

The coarse mesh DEAE cellulose, 8.5 g at 0.87 meq/g, was prepared for chromatography according to Methods in Enzymology (9), and packed in a 2.0 cm i.d. glass column. The column was equilibrated with 150-200 ml of the eluting buffer, and the chromatography was performed at room temperature.

The absorbance of each fraction was measured at 280 and 418 nm and those with the highest reading at 418 nm were selected for P-450 assay with CO and n-octylamine (NO). The method of Omura and Sato (10) was used to

obtain the CO·P-450 spectrum, treating the sample with CO prior to reduction with dithionite. The n-octylamine, 0.9-1.0 ul, was added directly to the sample cuvette (3.0 ml) from a 10 ul syringe. The resulting difference spectrum was recorded on an Aminco DW-2 spectrophotometer.

Results and discussion: The typical elution profile in Fig. 1 shows four major peaks with absorbance at 280 nm and three absorbing at 418 nm. The readings at 418 nm were used in following column behavior. Only traces of P-420 or P-450 eluted in the peak I (418 nm) region when solubilization was successful. Untreated or partially solubilized microsomes eluted as a turbid suspension in one column volume of the eluting buffer.

From the O.D. at 418 nm of the sample prior to chromatography, it was estimated that only 20-30% of the total light absorbing material was recovered from the columns. Apparently the majority of this material remained

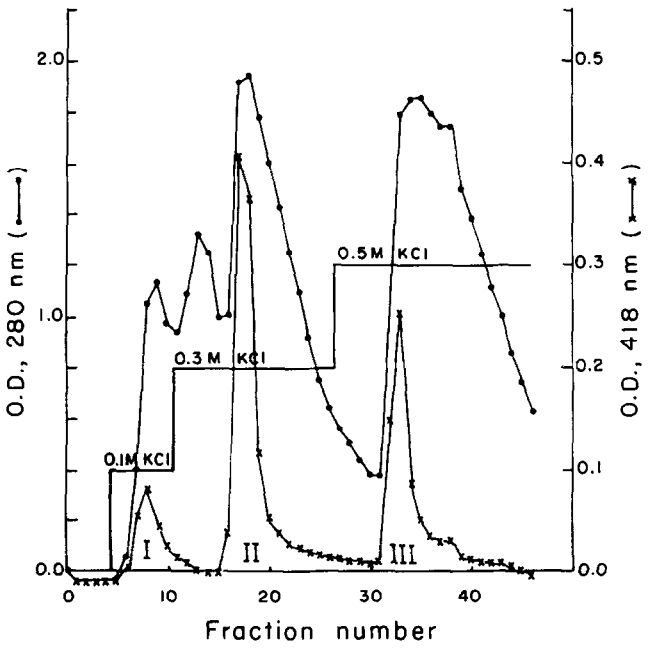

Figure 1. A typical elution profile obtained in the chromatography of deoxycholate-treated house fly microsomes on DEAE cellulose.

adsorbed on the cellulose. Buffer with KCl concentrations greater than 0.5M eluted additional 418 nm peaks but the fractions contained only small amounts of cytochrome P-420 and no P-450.

Assays of selected fractions for CO:P-450 showed that two forms of this enzyme were eluted from the column by the 0.3 and 0.5M KCl-buffer, peaks II and III, Fig. 1. Figure 2 shows that the spectra of the cytochrome in these 2 peaks differed in the λ_{max} of their CO:P-450 and in their relative contents of P-420. The λ_{max} from peak II was always 1 to 3 nm higher in wave length than that from peak III.

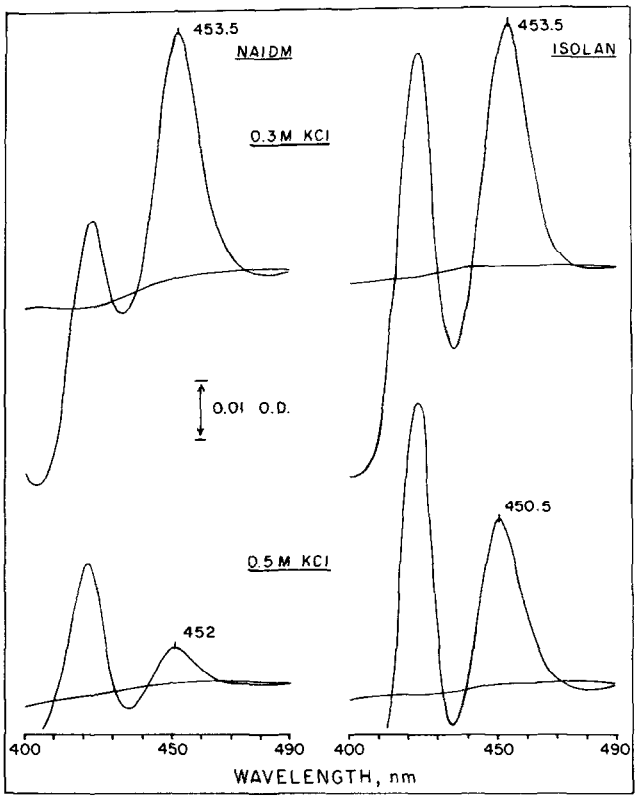

Figure 2. Representative CO:P-450 difference spectra of fractions eluting from DEAE cellulose chromatography of solubilized microsomes from two strains of house flies.

The Δ O.D. of the CO·P-420 was greater than that for P-450 in the fractions from peak III whereas the reverse was true for fractions from Peak II. This could be due to the inherent instability of the cytochrome eluting in 0.5M KCl-buffer or to an adverse effect of the higher salt concentration. Studies of the rate of formation of CO·P-420 and CO·P-450 in the two eluting regions provided evidence supporting the former possibility. The CO·P-450 spectrum from peak III reached its maximum O.D. in 5 minutes, with CO·P-420 continuing to increase, while CO·P-450 from peak II fractions increased in O.D. for as long as 30 minutes. The possible adverse effect of salt was checked by increasing the concentration of KCl in the peak II fractions to 0.5M and by diluting that of peak III to 0.3M. Neither condition affected the stability of the P-450.

The n-octylamine difference spectra in Fig. 3 also show that two forms of P-450 are present. This is seen in the shape of their trough and in

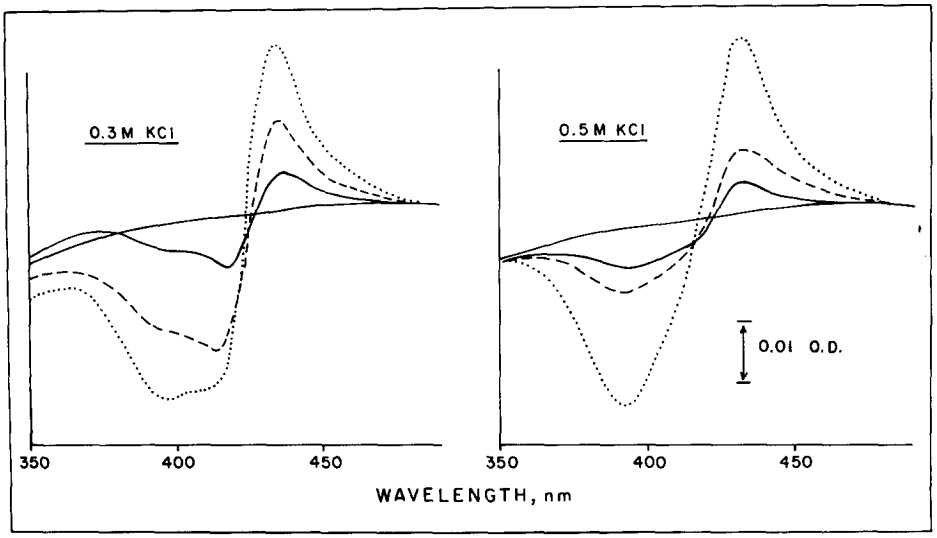

Figure 3. Representative n-octylamine difference spectra of fractions eluting from DEAE chromatography of solubilized microsomes from three strains of house flies: (—) SRS, (- -) Fc, (...) Isolan-B.

their λ_{max}. The complex formed by P-450 from Peak III had a minimum of 392 nm, an occasional inflection at 413 nm, and a λ_{max} at 431 nm, whereas that from peak II had the deeper trough at 413 nm and a λ_{max} at 434 nm. Similar NO spectral characteristics have been accredited to types a and b cytochrome P-450 and to high and low spin forms of the enzyme, respectively (11).

Both forms of P-450 were found in each of the house fly strains examined but there seemed to be more of the form eluted by 0.5 M KCl in the R strains than in the S strains (Fig. 2). There was no large difference in the amount of the peak II form in the four strains except for the low level in the SRS. The CO and NO spectra of the 2 forms of cytochrome P-450 isolated from the resistant Isolan-B strain, Figs. 2 and 3, coincide closely to such spectra already published for susceptible and resistant house fly strains (4, 12).

This new information about cytochrome P-450 in the house fly seems to answer most of the questions referred to earlier. It is clear that, if the microsomes of a house fly strain contain two P-450's, the measured CO·P-450 λ_{max} will be a composite of both forms of the enzyme, and its value could shift as much as 2-4 nm depending on the relative amounts of the forms present. Also, the instability of one of the forms could have a critical influence on the λ_{max} value obtained.

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