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PURIFICATION AND CHARACTERIZATION OF CYTOCHROME b_5 FROM THE HOUSEFLY *(MUSCA DOMESTICA)*

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Abstract—1. Isopropanol treatment of *Musca domestica* (house fly) microsomes extracted cytochrome b_5 and resulted in a minimum 5.2-fold purification.

2. Cytochrome b_5 was highly purified from the isopropanol extract using ion exchange and reverse phase high performance liquid chromatography.

3. A polyclonal antiserum was produced against the purified cytochrome b_5.

4. Purified house fly cytochrome b_5 had a molecular mass of 19.7 kDa, slightly higher than those reported for rabbit and rat microsomal cytochrome b_5 (16.6–16.7 kDa), but lower than cytochrome b_5 from Mediterranean fruit fly (21 kDa).

5. Reduced house fly cytochrome b_5 absorbed maximally at 424 nm, similar to other cytochromes b_5.

6. Amino acid analysis of house fly cytochrome b_5 produces a higher percentage of hydrophobic amino acid residues and less acidic residues than other cytochromes b_5.

7. In immuno-blot analysis, house fly cytochrome b_5 shared antigenic epitope(s) with the dipteran species *Musca autumnalis* (face fly), *Stomoxys calcitrans* (stable fly) and to a lesser extent, *Drosophila melanogaster* (fruit fly).

8. Anti-house fly cytochrome b_5 did not recognize cytochrome b_5 from *Apis mellifera* (honey bee), *Tricoplusia ni* (cabbage looper), *Tetranychus urticae* (two-spotted spider mite) or *Rattus rattus* (rat).

INTRODUCTION

In the endoplasmic reticulum, cytochrome b_5 transfers reducing equivalents from cytochrome b_5 reductase or cytochrome *P*450 reductase to stearyl-CoA desaturase or to cytochrome *P*450, and thus is thought to participate in many important cellular oxidation reactions (Estabrook, 1978). However, its role as a donator of the second electron to cytochrome *P*450 remains unresolved (Hildebrandt and Estabrook, 1971).

The ability to isolate and study intact cytochrome b_5 from insects is desirable since it is abundant in insects and may be involved with monooxygenase-dependent insecticide resistance (Scott *et al.*, 1990). House flies are an exceptionally good model to study the role of insect cytochrome b_5 in monooxygenase reactions since other components of the system are well studied in house flies (Ronis and Hodgson, 1989).

Intact cytochrome b_5 was first isolated from mammalian microsomes by Spatz and Strittmatter (1971), and several similar procedures for isolation were subsequently developed for mammalian tissues (Ozols, 1974; Carlsen *et al.*, 1988; Burchell, 1985). However, alternative procedures are required for house fly cytochrome b_5 (Wheelock and Scott, 1989). Proteolytically solubilized cytochrome b_5 was purified from armyworm (McFadden *et al.*, 1979). Intact (i.e. solubilized with detergent) cytochrome b_5 has been purified to apparent homogeneity from only two insects, the Mediterranean fruit fly *Ceratitis capitata* (Megias *et al.*, 1986) and the house fly. In the case of the house fly, cytochrome b_5 was isolated in small yield as a by-product of cytochrome *P*450 isolation (Wheelock and Scott, 1989). The poor yield of cytochrome b_5 was primarily due to the initial extraction procedure that was designed to optimize isolation of cytochrome *P*450. The present study resulted from experiments to optimize purification of cytochrome b_5 from house fly microsomes in yields sufficient for antiserum production and characterization.

MATERIALS AND METHODS

Rearing and microsome preparation

House flies were reared as previously described (Wheelock and Scott, 1989). Face flies, stable flies, fruit flies, honey bees, cabbage loopers and mites were obtained and reared as described by Wheelock *et al.* (1991). Male Wistar rats were obtained from Cornell research animal resources, Cornell University, Ithaca, NY.

Microsomes were prepared from house fly abdomens as previously described (Wheelock and Scott, 1990). Microsomes from other insects and mites were prepared as described by Wheelock *et al.* (1991). Rats were anesthetized and killed with carbon dioxide and microsomes prepared from the rat livers according to Wheelock and Scott (1990). All microsomes were resuspended in 5 ml phosphate buffer plus 20% glycerol (Wheelock and Scott, 1989) and stored at −80°C.

Assay of cytochrome b_5

Cytochrome b_5 was assayed according to Omura and Sato (1964) using sodium dithionite as reducing agent according to Capdevila and Agosin (1975). Cytochrome b_5 was

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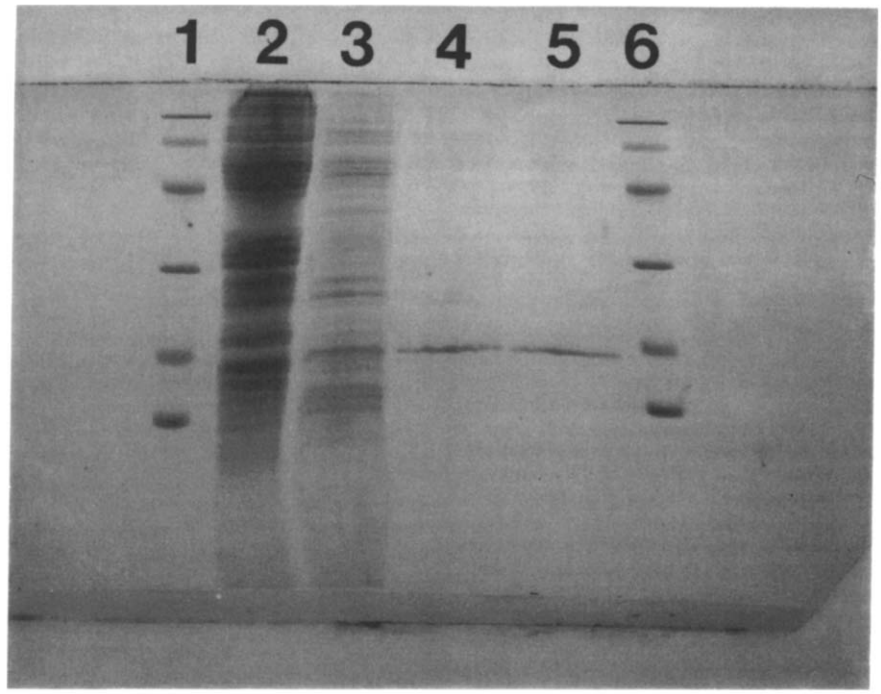

Fig. 1. Denaturing gel electrophoresis. Lane 1, 6: molecular mass markers (top to bottom 99.7, 66, 45, 31, 21.5, 14.4 kDa). Lane 2: 50 pmol crude microsomal cytochrome b_5. Lane 3: 50 pmol cytochrome b_5, isopropanol extract. Lane 4: 100 pmol cytochrome b_5, ion exchange HPLC fraction. Lane 5: 100 pmol cytochrome b_5, reverse phase HPLC fraction.

always assayed in the presence of detergent. Crude microsomes were solubilized in 0.5% CHAPS (3-[(3-cholamidopropyl)-dimethylammonio]-1-propanosulfanate, Sigma, St Louis, MO) or 0.4% emulgen (emulgen 911, Kao Atlas, Tokyo) prior to assay. Chromatography fractions contained 0.4% emulgen.

Extraction and isolation of cytochrome b_5

Five milliliters of LPR strain house fly microsomes were diluted with an equal volume of isopropanol (HPLC grade, Fisher Scientific, Rochester, NY) and immediately mixed. The cytochrome b_5 was extracted by gentle rocking at 4°C for 10 min, centrifuged at 8000 *g* for 10 min and the supernatant saved. The pellet was reextracted with 2 ml 50% isopropanol, 10% glycerol and 40% phosphate buffer and centrifuged again. The supernatants were pooled and diluted with an equal volume of 1.8×10^7 ohm deionized water, frozen to −80°C and lyophilized overnight.

The lyophilized extract was diluted to 4.2 ml with 0.4% emulgen in water, filtered through a 0.45 μm HPLC filter, divided in half, and chromatographed on a DEAE 5PW ion exchange high performance liquid chromatography (HPLC) column (Altex, San Ramon, CA) in two runs using conditions described previously (Wheelock and Scott, 1989). Cytochrome b_5 from the DEAE 5PW HPLC step was injected onto a VYDAC C8 reverse phase HPLC column (Alltech, Deerfield, IL) in two runs and developed using conditions described previously (Wheelock and Scott, 1989).

Antisera production

Polyclonal anti-house fly cytochrome b_5 was produced in a Flemish Giant/Chinchilla cross rabbit (Cornell research animal resources). For each immunization, purified cytochrome b_5 from the reverse phase HPLC step (see above) was diluted 25- or 50-fold with phosphate buffer containing 20% glycerol and 0.4% emulgen and concentrated on a YM2 filter (Amicon, Danvers, MA) to approximately 0.5 ml. The purpose of this step was to dilute out the isopropanol in the HPLC fraction prior to immunization. Concentrated antigen was diluted with an equal volume of Freund's adjuvant and injected subcutaneously at multiple sites along the rabbit's back. Freund's complete adjuvant was used for initial immunization and Freund's incomplete adjuvant was used for subsequent injections. Immunization schedule was as follows: 3.30, 3.83, 3.83 and 3.60 nmol purified cytochrome b_5 on days 1, 14, 29 and 64 respectively followed by exsanguination on day 71. The IgG fraction was paritally purified from the serum by two ammonium sulfate precipitations (Kunkel, 1988).

The anti-cytochrome b_5 antiserum was made monospecific (adsorbed) using the following procedure. Molecular mass markers (Biorad, Danvers, MA) and protein from 3 mg of house fly microsomes were resolved by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) using a 10% gel according to Laemmli (1970). Protein was transferred to a nitrocellulose sheet according to Towbin *et al.* (1979) with the specific conditions of Wheelock and Scott (1990). Extreme left and right ends of the sheet, containing the molecular mass markers, were removed and stained with Amido Black 10B to estimate the position of cytochrome b_5. Under these conditions house fly cytochrome b_5 has an apparent molecular mass of 19.7 kDa and runs near the bottom of the gel (Wheelock and Scott, 1989). The lower portion of the sheet containing the cytochrome b_5 was discarded and the remaining sheet was rinsed three times in phosphate buffered saline (Wheelock and Scott, 1990) and incubated with 0.5 ml antiserum at room temperature in a sealed plastic bag. After 4 hr the unbound, absorbed antiserum was recovered, combined with a 0.5 ml phosphate buffer rinse and stored at 4°C with 0.01% thimerosal.

Amino acid analysis

Purified cytochrome b_5 fractions (0.37–1.9 nmol) from reverse phase HPLC described above were diluted 1:1 with deionized water and lyophilized overnight in a Speed Vac Concentrator (Savant, Farmingdale, NY). Glycerol and

Table 1. Recovery of cytochrome b_5

Fraction	Yield nmol b_5	% Recovery at each step
Crude microsomes	7.04 ± 0.89	100%
Isopropanol extract	3.82 ± 0.56	54.4 (47–60)
DEAE 5PW HPLC	2.60 ± 0.30	75.2 (65–80)
C8 HPLC	2.33 ± 0.14	93.6 (88–100)

Yields are averages ± SD from five separate purifications using 5 ml microsomes starting material each, uncorrected for samples removed at each step for assays. Recoveries are average % recovery after that step compared with the activity found at the end of the previous step corrected for removal of material for assaay. Values in parentheses are ranges.

emulgen were removed from the residue by three washes of 4 ml ethyl ether. The remaining cytochrome b_5 was dissolved in 100 μl 1:2 85% formic acid: absolute ethanol and analyzed for amino acids using the Waters Pico-Tag System®. Separate analyses in the presence of thiodiglycol were done to measure tryptophan. In two out of four analyses, a large peak, tentatively considered to be unremoved emulgen, obscured CYS and/or ILE peaks. CYS and ILE values reported by the integrator were discarded from these analyses. All analyses were done by the Biotechnology Analytical and Synthetic Facility at Cornell University, Ithaca, NY.

Other methods

Protein in chromatography fractions was assayed using the detergent-tolerant method of Dulley and Grieve (1975). For analysis of purity and immuno-blotting, SDS–PAGE was performed according to Laemmli (1970) in 15% gels. Chromatography fractions, too ionic or too dilute for SDS–PAGE, were concentrated by precipitation in 10% ice-cold trichloroacetic acid, resuspension in lysis buffer (Laemmli, 1970) and neutralization with 1 M Trizmahydroxide (Sigma). Under these conditions emulgen in the chromatography fractions co-precipitated with cytochrome b_5 and trapped excess trichloroacetic acid, which therefore required excess neutralization and the reintroduction of ions. This produced abnormally broad bands in SDS–PAGE with the chromatography fractions. Electrophoresed samples for immuno-blot analysis were transferred to nitrocellulose according to Towbin *et al.* (1979). Nitrocellulose sheets were probed with absorbed anti-cytochrome b_5 antiserum at 1:500 dilution using the conditions of Wheelock and Scott (1990). Rocket immuno-electrophoresis was performed according to Wheelock and Scott (1990) with the following changes: agarose contained 0.5% CHAPS (Sigma) and 1.5% (unabsorbed) or 5.0% (absorbed, diluted) antiserum.

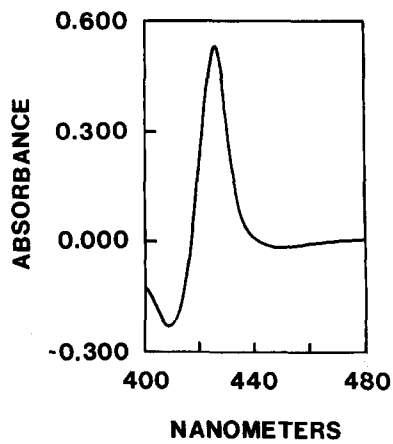

Fig. 2. Amicon membrane concentrated, purified cytochrome b_5 (4.15 nmol/ml). Scan represents the difference spectra between oxidized and reduced cytochrome b_5.

Table 2. Amino acid composition of house fly cytochrome b_5

Residue	Number
ASX	14
THR	11
SER	13
GLX	17
PRO	7
GLY	11
ALA	12
VAL	16
CYS	0
MET	8
ILE	10
LEU	12
TYR	5
PHE	11
HIS	3
LYS	18
ARG	4
TRP	0

ASX + GLX: 18%
HIS + LYS + ARG: 14.5%
APOLAR: 42.2%

Average of four determinations. TRP: average of two determinations done separately. CYS: two determinations. ILE: three determinations. Apolar residues: MET, VAL, LEU, ILE, PRO, TRP, PHE, ALA.

RESULTS

Extraction of cytochrome b_5

We have previously shown that house fly cytochrome b_5 is eluted in 38% isopropanol by C8 reverse phase HPLC and can be directly assayed when diluted 1:1 with phosphate buffer (Wheelock and Scott, 1989). This suggested to us that cytochrome b_5 would be soluble in, and recoverable from, high concentrations of isopropanol. In the present study, we found that extraction of the microsomes in 50% isopropanol resulted in an apparent 51.3% recovery of cytochrome b_5 (n = 10, range = 43–62). Additionally, the spec. act. of the isopropanol extract was 2.29 ± 0.068 nmol cytochrome b_5/mg protein (n = 5), compared with a specific content of 0.438 ± 0.053 (n = 5) for crude microsomes. This implies a 5.2-fold purification by isopropanol extraction compared with the detergent solubilized dithionite assayed crude microsomes. However, these values are most likely conservative, since assay of crude house fly microsomes with dithionite results in overestimation of cytochrome b_5 due to cytochrome *P*450 reduction (Estabrook and Werringloer, 1978; Ozols, 1974). In crude LPR microsomes, assay of cytochrome b_5 results in up to a 2-fold overestimation while partially purified cytochrome b_5 fractions (which do not contain cytochrome *P*450) are not subject to the same artifact (unpubl. observns). Thus, isopropanol extraction of cytochrome b_5 yields may approach 100% with a purification of about 10-fold. Furthermore, SDS–PAGE analysis of the extract showed significant enrichment for cytochrome b_5, suggesting that this was a suitable method for optimization of cytochrome b_5 purification from house flies (Fig. 1).

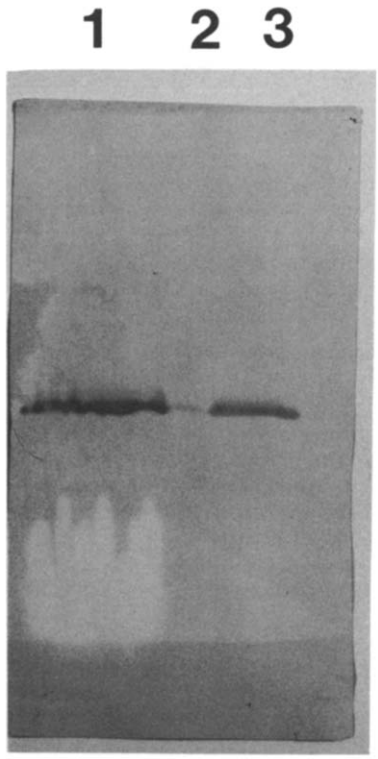

Fig. 3. Immunological detection of cytochrome b_5. Lane 1: 50 pmol purified cytochrome b_5. Lane 2: pellet after isopropanol extraction of microsomes, equivalent to 50 pmol cytochrome b_5 in original sample. Lane 3: 50 pmol cytochrome b_5, crude microsomes.

Purification of cytochrome b_5 *from isopropanol extract*

Isolation of house fly cytochrome b_5 as a by-product of cytochrome *P*450 purification consists of phenyl 5PW hydrophobic interaction HPLC, DEAE 5PW ion exchange HPLC, followed by C8 reverse phase HPLC (Wheelock and Scott, 1989). When the isopropanol extract of microsomes was used as the starting material, almost complete purification was achieved with only ion exchange HPLC and apparent homogeneity was achieved after reverse phase HPLC (Fig. 1). Thus, the original hydrophobic interaction HPLC step could be eliminated. Retention times of isopropanol-extracted cytochrome b_5 on DEAE–5PW and C8 HPLC were identical with those previously reported (Wheelock and Scott, 1989) and are not reported here. Recoveries of cytochrome b_5 were high throughout the isolation procedure, allowing a yield of 2.33 nmol of cytochrome b_5 per run (Table 1), 2.7-fold higher than previous results (Wheelock and Scott, 1989). Purified cytochrome b_5 showed an absorbance peak typical of cytochrome b_5 at 424 nm (Fig. 2) as found in crude house fly microsomes (Lee and Scott, 1989). This shows the isolated material was, in fact, cytochrome b_5, and that the cytochrome b_5 heme is not removed using this procedure (Burchell, 1985). Amino acid analysis of house fly cytochrome b_5 showed the following percentages of amino acids: 42.2% apolar (MET, VAL, LEU, ILE, PRO, TRP, PHE, ALA), 18% acidic (taken as ASX and GLX) and 14.5% basic (HIS, LYS, ARG). There were no cysteine or tryptophan residues detected (Table 2). *N*-Terminal amino acid analysis was unsuccessful due to *N*-terminal blockage (data not shown). The molecular mass of house fly cytochrome b_5 was estimated at 19.7 kDa (Fig. 1) in confirmation of our previous determination of 19.7 ± 0.76 using a different purification technique and SDS–PAGE system (Wheelock and Scott, 1989).

Anti-cytochrome b_5

The purified cytochrome b_5 from the reverse phase HPLC step was used as antigen to immunize a rabbit. The resulting antiserum reacted with cytochrome b_5 both as purified protein and microsomal cytochrome b_5 in immuno-blotting (Fig. 3). Immuno-blot analysis of microsomes from different animals revealed that the antiserum only recognized dipteran cytochrome b_5 among the types tested (Fig. 4). In order of decreasing band intensity, the antiserum recognized *Musca domestica* (LPR and S+ house fly strains), *Musca autumnalis* (face fly) and *Stomoxys calcitrans* (stable fly). In addition, the antiserum showed an extremely faint reaction with *Drosophila melanogaster* microsomes (Canton-S and Hikone-R strains). There were no reactions with microsomes from *Apis mellifera* (honey bea), *Tricoplusia ni* (cabbage looper), *Tetranychus urticae* (two-spotted spider mite) or *Rattus rattus* (rat). It should be noted that cytochrome b_5 was unassayable in mite microsomes. Although similar amounts of protein were loaded onto the gel compared with other samples, it is not known whether the lack of immuno-staining in this sample was due to lack of recognition by the antiserum or lack of an adequate amount of cytochrome b_5.

The absorbed antiserum did not form an immune precipitate with house fly cytochrome b_5 using rocket immuno-electrophoresis, unlike the unabsorbed antiserum (Fig. 5). Typically, rocket immuno-electrophoresis requires at least three to four epitope/antibody reactions per antigen to form the required insoluble immune-precipitate lattice. Conversely, immuno-blot analysis need only have one epitope/antibody reaction per antigen for a positive response (Kunkel, 1988). Therefore, the antigenic site(s) unique to house fly cytochrome b_5 and recognized by the absorbed antiserum are probably limited in number, since the absorbed antiserum did not form an immune precipitate while the unabsorbed antiserum did.

DISCUSSION

We report a procedure to extract house fly cytochrome b_5 in high yield that allows reduction of chromatographic steps needed to purify it from three to two, and increases the yield 2.7-fold over our previously reported procedure. We were able to isolate quantities sufficient to produce a polyclonal antibody to undamaged cytochrome b_5 holoprotein and to afford some preliminary characterization. Difficulties in producing an immune response in rabbits with cytochrome b_5 have been reported, especially with immunizations utilizing little protein (Carlsen *et al.*, 1988). Therefore, the significant

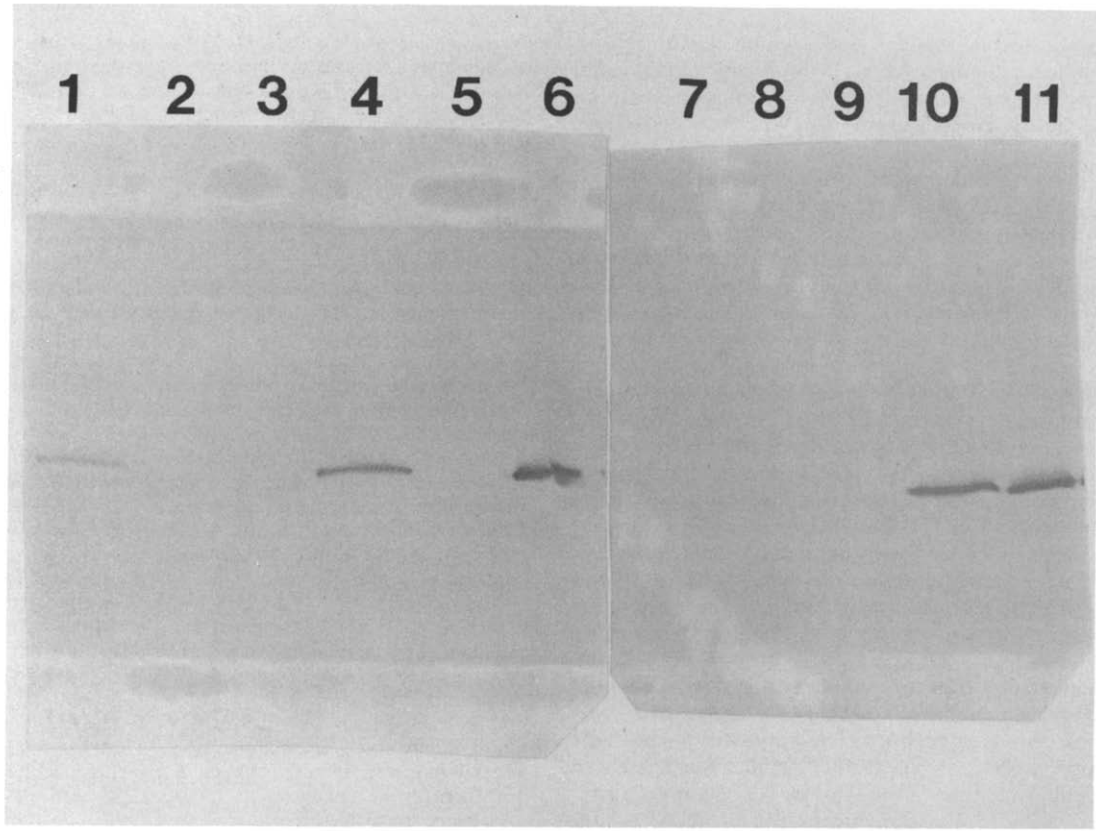

Fig. 4. Immuno-blot analysis of microsomal cytochrome b_5 from several animals. Microsomal loadings expressed as protein content: (1) *S. calcitrans*, 100 μg; (2) Canton-S strain *D. melanogaster*, 72 μg; (3) Hikone-R strain *D. melanogaster*, 74 μg; (4) *M. autumnalis*, 105 μg; (5) *A. mellifera*, 73 μg; (6) LPR strain *M. domestica*, 121 μg; (7) *T. ni*, 75 μg; (8) *T. urticae*, 75 μg; (9) *R. rattus*, 365 μg; (10) S + strain *M. domestica*, 143 μg; (11) LPR strain *M. domestica*, 121 μg. Cytochrome b_5 in *T. urticae* was unassayable. Other loadings contained 25 pmol cytochrome b_5 except (7) *T. ni* (8 pmol) and (9) *R. rattus*, which contained 50 pmol.

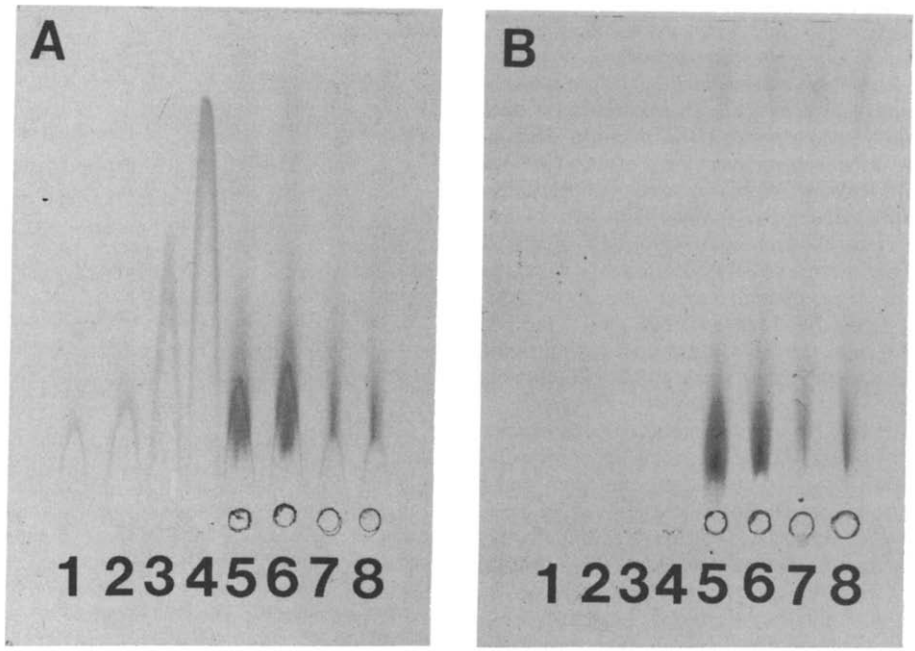

Fig. 5. Rocket immuno-electrophoresis of house fly cytochrome b_5. Slide A: unabsorbed antiserum. Slide B: absorbed antiserum. Picomoles purified cytochrome b_5 in parentheses: well 1 (1.5), well 2 (3.0), well 3 (6.0), well 4 (12). Picomoles crude microsomal cytochrome b_5: well 5 (5.2), well 6 (5.2), well 7 (2.6), well 8 (2.6).

improvement in yield and the demonstration of adequate immune response represent fundamental improvements over our previous technique for isolating house fly cytochrome b_5 (Wheelock and Scott, 1989).

The molecular mass for house fly cytochrome b_5 was previously reported as 19.7 ± 0.76 kDa (Wheelock and Scott, 1989) and was confirmed here as 19.7 (Fig. 1). The dipteran Mediterranean fruit fly cytochrome b_5 has a value of 21.0 kDa (Megias *et al.*, 1985), similar to house fly. In addition, immuno-blot analysis of stable fly and face fly also detected putative cytochromes b_5 at the same M_r as house fly cytochrome b_5. In contrast, mammalian cytochromes b_5 have masses in the range 16–19.0 kDa (Spatz and Strittmatter, 1971), with rabbit liver (16.7 kDa) and rat liver cytochrome b_5 (16.6 kDa) being typical (Spatz and Strittmatter, 1971; Burchell, 1985). Whether the higher masses seen with the dipteran cytochrome b_5 are typical of dipterans in general or of insects in general requires further study. The only other purified insect cytochrome b_5, that from armyworm (McFadden *et al.*, 1979), was proteolytically truncated so that the reported mass of 11.4 kDa does not represent the entire molecule.

House fly cytochrome b_5 appeared to be more hydrophobic, as judged by amino acid analysis (Table 2), than other insect or mammal cytochromes b_5. We have previously remarked on the unusually hydrophobic behavior of house fly cytochrome b_5 during size-exclusion chromatography (Wheelock and Scott, 1989). However, the difference in per cent apolar amino acids between house fly and other insect cyto- chromes b_5 was greater (42.2% vs 30.8–31.1%, respectively) than the same comparison between house fly and mammalian cytochromes b_5 (42.2% vs 34.3–39.7%, respectively) (Table 2, Megias *et al.*, 1985; McFadden *et al.*, 1979; Ozols, 1972, 1974; Spatz and Strittmatter, 1971).

The consistent richness of ASX and GLX residues compared with basic residues HIS, LYS and ARG in cytochromes b_5 has been noted by others (Megias *et al.*, 1985; Ozols, 1974). Compared with Mediterranean fruit fly (Megias *et al.*, 1985), armyworm (McFadden *et al.*, 1979), human (Ozols, 1972), rabbit (Spatz and Strittmatter, 1971), porcine, bovine and equine (Ozols, 1974) cytochromes b_5, house fly cytochrome b_5 had the lowest [ASX + GLX/HIS + LYS + ARG] ratio (i.e. 1.24, compared with a range of 2.13–2.48 for the insects and 1.41–1.76 for the mammals).

In summary, the molecular mass and absorbance maximum of house fly cytochrome b_5 are typical of other cytochromes b_5. However, the amino acid analysis reported here indicates that house fly cytochrome b_5 has somewhat unique characteristics compared with other cytochromes b_5. In addition, the lack of cross-reactivity between anti-house fly cytochrome b_5 and non-dipteran cytochrome b_5 (Fig. 4) also suggests divergence between fly cytochrome b_5 and other cytochromes b_5. Whether these characteristics translate into unusual functional properties for house fly cytochrome b_5 remains to be seen.

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REFERENCES

Burchell A. (1985) A simple method for purification of rat hepatic microsomal cytochrome b_5. *Biochem. J.* **226,** 339–341.

Capdevila J. and Agosin M. (1975) Soluble cytochrome *P*-450 from housefly microsomes. Partial purification and characterization of two hemoprotein forms. *J. biol. Chem.* **250,** 1049–1060.

Carlsen J., Christiansen K. and Jensen H. M. (1988) Rat hepatic microsomal cytochrome b_5. *Biochem. J.* **256,** 1051–1054.

Dulley J. R. and Grieve P. A. (1975) A simple technique for eliminating interference by detergents in the Lowry method of protein determination. *Analyt. Biochem.* **64,** 136–141.

Estabrook R. W. (1978) Microsomal electron-transport reactions: an overview. In *Methods in Enzymology* (Edited by Fleischer S. and Packer L.), Vol. 52, pp. 43–47. Academic Press, New York.

Estabrook R. W. and Werringloer J. (1978) The measurement of difference spectra: application to the cytochromes of microsomes. In *Methods in Enzymology* (Edited by Fleischer S. and Packer L.), Vol. 52, pp. 212–220. Academic Press, New York.

Hildebrandt A. and Estabrook R. W. (1971) Evidence for the participation of cytochrome b_5 in hepatic microsomal mixed-function oxidation reactions. *Archs Biochem. Biophys.* **143,** 66–79.

Kunkel J. G. (1988) Analytic immunologic techniques. *Immunological Techniques in Insect Biology* (Edited by Gilbert L. I. and Miller T. A.). Springer, New York.

Laemmli U. K. (1970) Cleavage of structural proteins during assembly of the head of bacteriophage T4. *Nature* **227,** 680–685.

Lee S. S. T. and Scott J. G. (1989) Microsomal cytochrome *P*450 monooxygenases in the house fly (*Musca domestica* L.): biochemical changes associated with pyrethroid resistance and phenobarbital induction. *Pestic. Biochem. Physiol.* **35,** 1–10.

McFadden K. A., Crankshaw D. L., Hetnarski H. K. and Wilkinson C. F. (1979) Purification and properties of cytochrome b_5 from the larval midgut of the southern armyworm (*Spodoptera eridania*). *Insect Biochem.* **9,** 301–308.

Megias A., Sabrido A. and Municio A. M. (1986) Cytochrome b_5 from the insect *Ceratitis capitata*. *Biochem. biophys. Acta* **872,** 116–124.

Omura T. and Sato R. (1964) The carbon monoxide binding pigment of liver microsomes. I. Evidence for its hemoprotein nature. *J. biol. Chem.* **239,** 2370–2378.

Ozols J. (1972) Cytochrome b_5 from a normal human liver. *J. biol. Chem.* **247,** 2241–2254.

Ozols J. (1974) Cytochrome b_5 from microsomal membranes of equine, bovine and porcine livers. Isolation and properties of preparations containing the membranous segment. *Biochemistry* **13,** 426–439.

Ronis M. J. J. and Hodgson E. (1989) Cytochrome *P*-450 monooxygenases in insects. *Xenobiotica* **19,** 1077–1092.

Scott J. G., Lee S. T. and Shono T. (1990) Biochemical changes in the cytochrome *P*450 monooxygenases of seven insecticide-resistant house fly (*Musca domestica* L.) strains. *Pestic. Biochem. Physiol.* **36,** 124–127.

Spatz L. and Strittmatter P. (1971) A form of cytochrome b_5 that contains an additional hydrophobic sequence of

40 amino acid residues. *Proc. natn. Acad. Sci. USA* **68,** 1042–1046.

Towbin H., Staehelin T. and Gordon J. (1979) Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. *Proc. natn. Acad. Sci. USA* **76,** 4350–4354.

Wheelock G. D., Konno Y. and Scott J. G. (1991) Expression of $P450_{lpr}$ in house flies. *J. Biochem. Toxic.* **6,** 239–246.

Wheelock G. D. and Scott J. G. (1989) Simultaneous purification of a cytochrome *P*-450 and cytochrome b_5 from the house fly, *Musca domestica* L. *Insect Biochem.* **19,** 481–488.

Wheelock G. D. and Scott J. G. (1990) Immunological detection of cytochrome *P*450 from insecticide susceptible and resistant house flies. *Insecticide Biochem. Physiol.* **38,** 130–139.