GEL ELECTROPHORESIS OF PARTIALLY PURIFIED CYTOCHROMES P_{450} FROM LIVER MICROSOMES OF VARIOUSLY-TREATED RATS

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SUMMARY

Liver microsomes and partially purified cytochromes P_{450} prepared from untreated animals or those injected with phenobarbital, or 3-methylcholanthrene, or polychlorinated biphenyls, were subjected to slab-gel SDS-electrophoresis. There were observed marked differences, after these treatments, in the gel-electrophoresis patterns of the induced cytochromes P_{450} in the microsomes and partially purified preparations.

The existence in liver endoplasmic reticulum (ER) of a cytochrome P_{450}-linked oxidation system which can metabolize a large and varied number of substrates has led to the concept that more than one mixed-function oxidase system occurs in these membranes, containing more than one species of cytochrome P_{450} (1). The treatment of rats with 3-methylcholanthrene (MC) results in the induction of cytochrome P_{448}, a hemeprotein that differs in spectral and catalytic activities from cytochrome P_{450} present in untreated rats or in rats treated with phenobarbital (PB, 2-5). Recent studies have shown that polychlorinated biphenyls (CB) share the properties of both the MC and the PB type of inducer compounds and the hemeprotein induced by treatment of rats with CB may be a mixture of cytochromes P_{448} and P_{450} exhibiting catalytic properties of both cytochromes (6). It has also recently been shown (7) that three spectrally-distinguishable forms of cytochrome P_{450} can be separated on DEAE-cellulose after detergent solubilization of microsomes.

It thus becomes of interest to know if different species of cytochrome P_{450} could be found in the microsomes of these differently-treated rats. Indeed, it has been found (8) that a comparison of the SDS-gel electrophoresis profiles of ER membrane proteins from rats injected with PB and those injected

with MC showed a difference in stained peaks in the 50,000 MW region. It has previously been shown (9-11) that solubilization of partially purified cytochrome P_{450} by SDS results in polypeptide(s) with MW close to 50,000. We therefore decided to repeat the earlier experiments (8) but with a gel system of higher resolution and to extend them to microsomes and partially purified cytochrome P_{450} preparations from normal, PB-, MC- and CB-injected animals, all of the latter three conditions causing a large increase in the cytochrome P_{450} contents of liver microsomes. The results, showing a marked difference in gel-electrophoresis patterns among the preparations, will be discussed in relation to the structure of cytochrome P_{450}.

MATERIALS AND METHODS

Male Sprague-Dawley rats weighing 50-60 g were used. The CB mixture used was Aroclor 1254 supplied by Monsanto Chemical Co., St. Louis, Mo. CB, dissolved in corn oil, was administered i.p. at a dosage of 25 mg/kg/day for 6 days. PB and MC were administered by the same route at dosages of 75 mg/kg/day and 25 mg/kg/day, respectively for 4 days. Liver microsomal fractions were prepared and washed (12), and the partial purification and solubilization of the cytochrome P_{450} fractions were carried out by the method of Lu and Levin (12). Cytochrome P_{450} content was determined by the method of Omura and Sato (13) using an extinction coefficient of 91 mM^{-1} cm^{-1} for $A_{450-490}$. Protein was determined by the method of Lowry *et al.* (14).

The microsomal suspensions, consisting mostly of ER membranes, were mixed with the membrane-protein dissolving agent, SDS, to give a final protein concentration of ~ 10 mg/ml in 2% SDS, 0.05 M Na_2CO_3 and 10% β-mercaptoethanol. The clarified suspensions were spun at low speed to remove the very small amount of undissolved protein. Purified cytochrome P_{450} preparations were also mixed with the SDS solution to give the same final concentrations as above. The gel electrophoresis was done according to the discontinuous buffer system of Neville (15) except that a slab gel was used. These slab gels were made using a gradient-forming apparatus so that the concentration of total

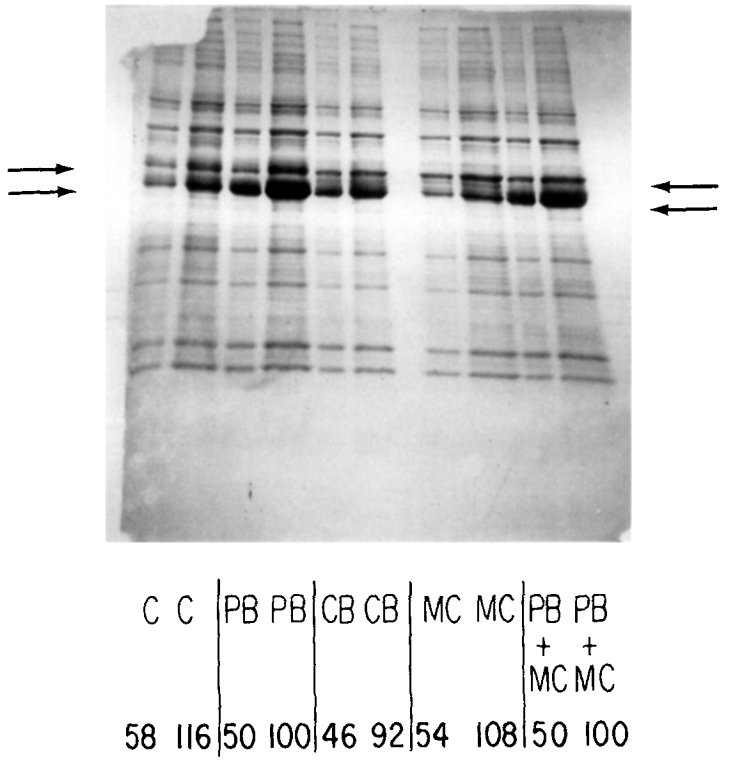

Fig. 1 Slab-gel electrophoresis of rat liver microsomal membrane proteins from variously-treated animals. Injection of the animals, preparation of the microsomes, and the gel electrophoresis were performed as described in Methods. C, PB, CB, and MC refer to control, phenobarbital-, chlorinated biphenyl-, and methylcholanthrene-injected animals; the figures beneath (in μg), refer to the amount of protein put on the gels. The amount of cytochrome P_{450} (nmoles/mg protein) in the microsomal preparations were as follows: C, 0.6; PB, 1.9; CB, 1.9; MC, 1.6. The arrows at the left denote the positions of 47,000 and 52,000 MW proteins; origin is at the top.

acrylamide in the separation gel varied linearly from 7.5 to 15% and that in the stacking gel was kept at 5%. The gradient was made so that of the total acrylamide, 2.6% was methylenebisacrylamide all throughout the separating gel and in the stacking gel. Also, the amount of ammonium persulfate was reduced to 0.025% to allow for a longer time for polymerization. The pH's of the lower reservoir and lower gel buffer, of the upper reservoir buffer, and of the upper gel buffer were respectively 9.18, 8.64 and 6.10. Electrophoresis was carried out for ~ 12 hr at 10 mamp/gel at room temperature, until the tracking dye, bromphenol blue at a final concentration of 0.001% in the sample, reached the

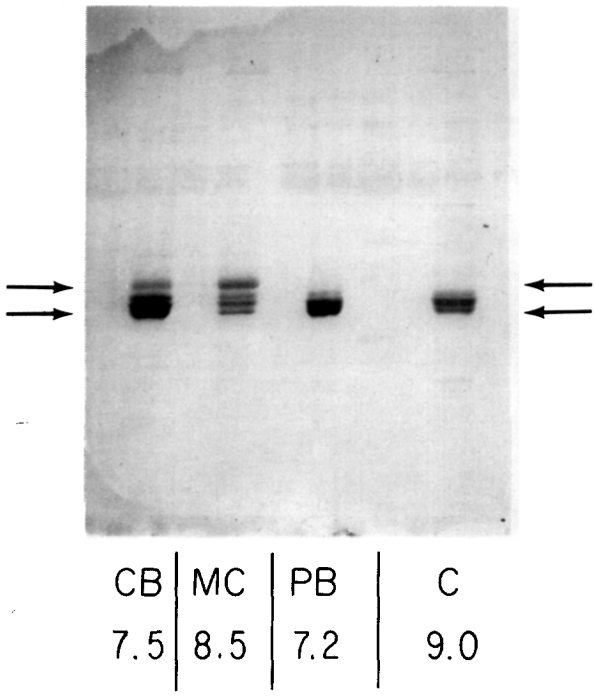

Fig. 2 Slab-gel electrophoresis of purified cytochrome P_{450} preparations of rat liver microsomes from various animals. Methods as in Fig. 1; abbreviations and figures beneath, same as in Fig. 1. The amount of cytochrome P_{450} (nmoles/mg protein) in the purified preparation was as follows: C, 1.3; PB, 4.7; MC, 3.6; CB, 4.4. The arrows at the left denote the positions of 47,000 and 52,000 MW proteins; origin is at the top.

bottom of the slab. The gels were fixed and stained at the same time, by incubating at room temperature for 3 hr in a solution of 0.25% Coomassie blue in 50% methanol-7% acetic acid. Excess dye was removed by extensive washing with 30% methanol. Molecular weight markers and comparisons were done as described earlier (11).

RESULTS AND DISCUSSION

Fig. 1 shows a comparison of SDS-gel electrophoresis profiles of microsomal membranes from control rats and from animals injected with PB, MC, CB and a mixture of PB and MC. It is clear that the only difference among them is the large variation in the 47,000 to 52,000 MW regions. These variations are of two kinds: 1) the injected animals all show an increase in staining only in this region, as has previously been found for PB-injected animals (10,11), and

which finding has been used to identify the polypeptides in this region as being cytochrome P_{450} since the content of cytochrome P_{450} is also increased in the microsomes obtained from these injected animals; 2) the relative proportion of the various bands in this region is varied among the microsomes from the differently-treated animals. As to the latter case, in the control animals, three bands of molecular weights 47,000, 49,000, and 52,000 are visible with possibly a fourth band, at 51,000. After PB-injection, it would appear that only the bands at 47,000 and 49,000 are increased; after MC-injection only the band at 52,000 is increased; after PB- plus MC-injection all the bands seem to be increased, and after CB-injection again all the bands seem to be increased. Levin et al. (9) found 50,000 and 59,000 MW bands in the control rats; both bands were increased after PB-injection and after MC-injection only the 50,000 MW band was increased. Welton and Aust (8) found that several bands occurred in the region and that after PB-induction, a 49,000 MW band was increased while after MC-induction a 53,000 MW band was increased. Our results thus also show a difference in the relative amounts of stained bands in this region, and we agree in that we find that after PB-injection lower MW bands (47,000, 49,000) are specifically increased, while after MC-injection, a higher MW band ($\sim$ 52,000) is specifically increased. In the case of CB-injection all these bands seem to be increased, giving a band pattern very similar to that found after a mixture of PB and MC was injected. It is intriguing that the oxidase and spectral properties of cytochrome P_{450} from CB-injected animals resemble a combination of the properties of the cytochrome P_{450} from separately injected MC and PB animals (6); thus the gel electrophoretic patterns of the polypeptides in the cytochrome P_{450} region seem to be a reflection of the catalytic properties of the cytochrome P_{450} in the microsomes of these rats. This is also shown in Fig. 2 (cf. below).

The patterns in Fig. 2, showing the gel electrophoretic behavior of the cytochrome P_{450} purified from controls, PB-, MC- and CB-injected animals mostly confirm the findings found with whole membrane preparations. The cytochrome

P_{450} preparations from control animals give four major bands, from 49,000 to 52,000, that from the PB-injected animals show a decided increase in the bands ~ 47,000 and ~ 49,000; that from the MC-injected animals show a selective increase in a band ~ 52,000, while that from CB-injected animals show an increase in the 47,000, 49,000 and 52,000 bands, a pattern again resembling an additive combination of the cytochrome P_{450} preparations from MC- and from PB-injected animals. A comparison thus of the purified cytochrome P_{450} preparations with the membrane fractions from both the MC- and CB-injected animals show that all four bands, from 47,000 to 52,000 are present. However, the purified cytochrome P_{450} preparations from the control and from the PB-injected animals have seemingly lost the heavier, ~ 52,000 MW, band; even higher amounts (14 μg) placed on the gel failed to show this band. Since the purification of the cytochrome P_{450} from the membranes is the same in all preparations, our tentative conclusion regarding the loss of bands is that all the four bands are part of the P_{450} complex, but that purification of the complex from controls and PB-injected animals results in a loss of one band, while all the bands are retained in the cytochrome P_{450}'s purified from the MC- and from the CB-injected animals.

What is the nature of these at least four bands? Our hypothesis is based on the recent findings that a somewhat purified cytochrome P_{450} complex isolated from rat liver microsomes has an apparent MW of 350,000 (16). It could be that the at least four bands represent subunit polypeptides of this complex, that only one or some of these subunits is the heme binding subunit, that the others bind various types of substrates differentially and that the different oxidase and spectral properties of the cytochrome P_{450} preparations obtained from rats after various injection conditions reflect a differential increase in some of these subunits relative to the others. It is conceivable that a change in the relative amounts of these subunits in a cytochrome P_{450} complex could lead to differences in the spectral properties of the heme bound to one of them and to the differences in the ability of various preparations to oxidize different substrates.

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