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NUTRITIONAL INFLUENCE ON SOME CYTOCHROME P-450 CHARACTERISTICS

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Alterations in the composition of diet influence the liver levels of total cytochrome P-450 as well as the activities of microsomal monooxygenases in the rat (1-3). It is conceivable then that, in addition to xenobiotics, imbalances in the relative amounts of normal food constituents may differentially influence the pattern of the various cytochrome P-450 isoenzymes. In this study we have compared the primary structure and activities of the purified forms of cytochrome P-450 from adult male Sprague Dawley rats fed for 6 weeks either a diet containing 6 % casein (low protein diet LP) or a diet containing 22 % casein (standard diet St).

Methods

Liver microsomal cytochrome P-450 was solubilized with sodium cholate and purified by affinity chromatography on w-n-amino-octylsepharose 4B prepared as described (4). Cytochromes were eluted with Emulgen 911, dialyzed overnight against phosphate buffer and then applied on a Whatman DEAE-52 cellulose ion exchange column. The column was washed with a linear gradient of sodium chloride and two cytochromes were sequentially eluted with the apparent minimum molecular weights of 50,000 and 52,000 as determined by SDS polyacrylamide gel electrophoresis (5). The preparations contained 15-17 nmol cytochrome P-450 per mg protein and gave a single band on SDS gel electrophoresis. The cytochrome P-450 forms were identified in Ouchterlony double diffusion experiments, and Elisa test by using antisera prepared against the purified cytochromes P-450 (6). Conditions used for the reconstitution of monooxygenase activity consisted of, per milliliter, 0.5 nmol cytochrome P-450, 50 units of cytochrome P-450 reductase and 0.1 mg dilauryl phosphatidyl choline (7). A direct fluorimetric assay for 7-ethoxycoumarin (8), a spectrophotometric assay for ethylmorphine demethylation (9) and the radiometric assay for benzo(a)pyrene hydroxylation (10) were used. Amino acid analysis was performed on the purified cytochromes after 5.7 N HCl hydrolyses at 110°C for 24 and 72 hours by using a Contron analyzer (11). Tryptophan and cystein values were determined separately (12, 13).

Results and Discussion

In Ouchterlony double diffusions experiments, and Elisa test antisera prepared against the purified cytochromes from St group crossreacted with microsomal proteins from rats fed with the LP diet. But all catalytic activities of isoenzymes purified from the LP group were lower than those purified from the St group. The diminished activity was more apparent for the lower molecular weight cytochrome (-50 % for benzo(a)pyrene hydroxylase and -83 % for ethylmorphine demethylase) than the higher molecular weight cytochrome (-25 % and -35 % respectively for the two activities). The lower molecular weight cytochrome purified from the low protein group, had 48 % hydrophobic amino acid residues (as compred to 40 % for the cytochrome purified from the St group) with the amino acids isoleucine, leucine and phenylalanine most dramatically increased. The positively/negatively charged amino acid ratio increased from 1.1 for the cytochrome from the St group to 1.36 for that from the LP group. The amino acid composition of the higher molecular weight cytochrome

was not significantly affected by diet. The structure-catalytic activity relationship are summarized in Fig. 1. For the lower molecular weight cytochrome both the hydrophobic/polar amino acid and positively/negatively charged amino acid ratios were increased by the low protein diet. This significant structural alteration corresponded to a strong decrease in catalytic activity. For the higher molecular weight cytochrome less dramatic changes in amino acid ratios corresponded to little decrease in catalytic activity. This study shows that indeed nutritional imbalances are sufficent to change the pattern of hepatic cytochromes P-450 in rat liver.

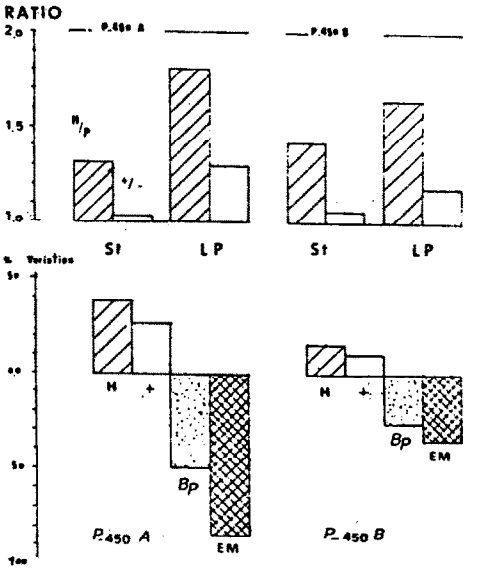

Fig. 1 Effect of low protein diet on the structure and catalytic activity of purified forms of liver cytochrome P-450

P-450 A = cytochrome P-450 with 50,000 MW, P-450 B = cytochrome P-450 with 52,000 MW.

H/P Hydrophobic/Polar amino acids ratio

+/- positively/negatively charged amino acid ratio

St cytochrome from rats fed a standard diet

LP cytochrome from rats fed a low protein diet

H % difference in hydrophobicity between cytochromes from LP and St groups

\+ % difference in positive charge of cytochromes from LP and St groups

Bp % difference in benzo(a)pyrene hydroxylase activity (nmol product per nmol cytochrome P-450 per min) of cytochromes from LP and St groups

EM % difference in ethylmorphine demethylase activity (nmol product per nmol cytochrome P-450 per min) of cytochromes from LP group and St groups.

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